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Journal of the Fisheries Research Board of Canada

Effects of Sodium Chloride on Outgrowth and Toxin Production of *Clostridium botulinum* Type E in Cod Homogenates

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Boyd, J. W., and B. A. Southcott. 1971. Effects of sodium chloride on outgrowth and toxin production of *Clostridium botulinum* type E in cod homogenates. J. Fish. Res. Bd. Canada 28: 1071–1075.

The sodium chloride tolerance of *Clostridium botulinum* type E strains Minnesota, Saratoga, and Tennessee was determined in heat-processed cod homogenates prepared from fresh frozen Pacific cod (*Gadus macrocephalus*). Homogenates were inoculated with heat-activated spores and incubated anaerobically at 30 C. The Minnesota strain produced a maximum toxin titer of 800 MLD (minimal lethal dose)/g in homogenates without added NaCl (control samples); a brine concentration of 3.76% (NaCl content in aqueous phase) inhibited toxin formation in homogenates, but did not inhibit outgrowth and vegetative cell multiplication. A maximum titer of 75 MLD was formed by the Saratoga strain in control samples; growth occurred in homogenates with a 3.84% brine content without the formation of toxin. The Tennessee strain produced a maximum titer of 1000 MLD in control samples; toxin of low potency was formed in homogenates containing 4.40% brine. A brine concentration of 5.50% in homogenates was sporistatic to the Saratoga and Tennessee strains, but not to the Minnesota strain. Toxin was produced in subcultures prepared from homogenates in which growth was inhibited by NaCl.

Received April 8, 1971

Clostridium botulinum type E spores are sensitive to various chemicals, such as sodium chloride, at certain stages of spore germination and vegetative cell development. Toxin formation in substrates inoculated with type E spores is also partially or completely inhibited by certain NaCl concentrations. The type of substrate used in tests to determine the NaCl tolerance of type E spores has some influence on outgrowth and toxin production.

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Investigators have reported the NaCl tolerance of type E strains in various substrates: bacteriological culture media (Abrahamsson et al. 1966; Segner et al. 1966; Baird-Parker and Freame 1967; Ohye et al. 1967; Riemann 1967; Emodi and Lechowich 1969), smoked fish (Kautter 1964; Sheneman 1965; Christiansen et al. 1968; Hayes et al. 1970), and meat products (Pivnick and Bird 1965; Warnecke et al. 1967). Bohrer et al. (1965) reported the NaCl tolerance of six strains of type E in homogenates prepared from fresh frozen chubs; fresh rather

than smoked chubs were used to obtain a more uniform substrate.

The objective of this present investigation was to determine the effect of NaCl concentration on growth and toxinogenesis of *C. botulinum* type E strains Minnesota, Saratoga, and Tennessee in heat-processed homogenates of Pacific cod (*Gadus macrocephalus*).

Materials and Methods

Cultures

Clostridium botulinum type E, strains Minnesota, Saratoga, and Tennessee were obtained from K. Ito, National Canners Association, Berkeley, California. Cultures were maintained in Cooked Meat Phytone medium (CMP), Baltimore Biological Laboratory (BBL); spore stocks were maintained in sterile soil.

Spore Suspensions

Spore suspensions were prepared by inoculating 1400-ml quantities of Trypticase (BBL), peptone, and glucose medium (TPG) (Schmidt et al. 1962) with 48-hr TPG cultures, heat activated 13 min at 60 C. Incubation was at 30 C in an anaerobic incubator with an atmosphere of 90% N_2 and 10% CO_2. After incubation for 2–3 days, the cultures were examined microscopically under dark phase to determine the extent of sporulation. When sporulation was greater than 80%, the broth cultures were stored at 4 C for 16 hr, and then centrifuged to collect spores. Spore suspensions were washed four times in sterile phosphate buffer, *p*H 7.2, and stored in buffer at 4 C.

Agar at a concentration of 0.65% (Eller et al. 1967) was added to Reinforced Clostridial Medium (RCM) (Oxoid) for the preparation of pour plates to ascertain the percentage viability of spores. Inoculated plates were incubated anaerobically at 30 C for 48 hr in a Case-Anaero Jar (Case Laboratory Inc., Chicago, Illinois); a small quantity of calcium chloride was placed in the jar to absorb excess moisture. Colonies were counted using a Quebec colony counter.

Preparation of Substrates

Homogenates, dilutions of comminuted fish muscle with an equal weight of water, were prepared from fresh-frozen fillets of Pacific cod as described by Boyd and Southcott (1968). Sodium chloride (NaCl) was added at various levels to portions of homogenate, and mixed thoroughly to provide even distribution of salt. Homogenates were dispensed in 100-g quantities into 6-oz screw-top jars, and thermal-processed at 116 C for 30 min.

RCM was used as the substrate in one test to ascertain the effect of NaCl concentration on the outgrowth in broth of spores of the Minnesota strain.

Moisture and Sodium Chloride Determinations

After homogenates were processed and cooled, a 25-g composite sample at each NaCl level was prepared from five jars. Percent moisture was determined on a moisture balance. Quantab chloride titrators (Ames) were used to determine NaCl levels; the results from representative samples with various NaCl levels were checked by flame photometry. The NaCl concentration is expressed in the results as percent brine: brine % = % NaCl in aqueous phase of homogenate.

Inoculation of Homogenates

The prepared fish homogenates were inoculated with heat-activated (60 C for 13 min) spore suspensions of Minnesota, Saratoga, or Tennessee strains to produce a final concentration of approximately 10^6 spores/g. Five replicate jars of homogenate were used at each NaCl level for each strain. The viability of spores was checked by inoculation of tubes of RCM. Inoculated homogenates were incubated at 30 C in an anaerobic incubator with an atmosphere of 90% N_2 and 10% CO_2. At intervals, homogenates were examined for evidence of growth.

Outgrowth and Toxin Assay

The term "outgrowth" in this report refers to all stages involved in vegetative cell development subsequent to spore germination. At intervals during incubation, homogenates were examined for evidence of aerogenesis, spore outgrowth, and toxinogenesis. Samples of homogenate were prepared as described by Boyd and Southcott (1968) for microscopic examination under dark-phase contrast to determine the extent of spore outgrowth.

For the determination of toxin production and potency in homogenates, a composite sample of approximately 30 g was prepared from the contents of five jars at each NaCl level; samples were thoroughly mixed with an equal weight of gelatin–phosphate buffer (*p*H 6.5) (Duff et al. 1956) and then stored at 4 C for 16 hr to ensure complete extraction of toxin. Samples were centrifuged, an aliquot of the supernatant was trypsin-digested (Duff et al. 1956), then 0.5 ml was injected intraperitoneally into each of two white mice weighing approximately 20 g. The toxin potency of positive samples was determined by dilution of supernatants with gelatin–phosphate buffer and was expressed as mouse minimal lethal dose (MLD) per gram of homogenate after death of two mice injected with the highest "toxic" dilution. Neutralization tests were carried out by injecting pairs of mice with 0.5 ml of a preincubated mixture (25 C for 30 min) of sample plus monovalent type E antitoxin. The mice were observed for 72 hr.

Results and Discussion

The results of toxin assays on cod homogenates inoculated with heat-activated spores of type E Minnesota strain are summarized in Table 1; all

TABLE 1. Effects of NaCl concentration on outgrowth and toxin production of *Clostridium botulinum* type E (Minnesota strain) in cod homogenates during incubation at 30 C.

Brine %	Moisture %	pH	Incubation (*days*)	Outgrowth	Toxin (*MLD/g*)
0	84.2	6.9	2	+[a]	220
			4		800
1.73	85.2	6.8	2	+	100
			4		115
2.50	85.0	6.8	2	+	0
			4		10
			6		100
2.96	85.2	6.7	2	–[b]	0
			4	+	10
3.40	85.0	6.7	4	+	<10
			6	+	0
			12	+	0
3.76	84.4	6.7	6	–	0
			10	+	0
			22	+	0

[a]Outgrowth.
[b]No outgrowth.

sampling times are not presented in the table. After 4 days incubation, the toxin potency in homogenates containing 2.50 and 2.96% brine was approximately 98% less than in control homogenates (NaCl not added). Outgrowth of germinating spores was initially delayed in samples containing 2.50 and 2.96% brine; direct microscopic observations of sample dilutions under phase contrast after 2 days incubation revealed that control samples contained a combination of vegetative cells (25%) and sporangia (75%) totalling 250 × 10^6/g, whereas the 2.50% brine sample contained germinating spores and a few vegetative cells, and the 2.96% brine sample contained refractile and germinating spores with no indication of outgrowth. Although a low toxin titer was found in 3.40% brine samples after 4 days incubation, toxin was not present in 6- and 12-day samples; the direct cell count of 6-day samples was 120 × 10^6/g consisting of vegetative cells and 15–20% sporangia. The toxin in the 6- and 12-day samples was either too low in concentration for assay or was destroyed by an unknown factor. A brine concentration of 3.76% in homogenates inhibited toxin production by the Minnesota strain. This is in accord with the results of Bohrer et al. (1965), who reported that growth of type E strains occurred without the production of toxin in fresh fish homogenates containing 3.79% brine. Results of tests that are not included in Table 1 revealed that a brine concentration of 5.50% (5.0% NaCl) did not inhibit growth of the Minnesota strain; toxin was not produced. Dolman (1964) reported that growth of a toxigenic type E strain was not inhibited in meat medium containing 5.0% added NaCl and that toxin was produced in significant amounts. Baird-Parker and Freame (1967) reported that 4.8% NaCl in RCM did not inhibit the growth of the type E Beluga strain. Riemann (1967) found that less than 4.0% NaCl in brain heart infusion broth did not inhibit outgrowth of spores of the Minnesota strain. Spore outgrowth of the Minnesota strain was inhibited in RCM with 4.0% added NaCl (B. A. Southcott and J. W. Boyd unpublished data).

The results of toxin assays on homogenates inoculated with spores of either the Saratoga or Tennessee strain are summarized in Table 2. Maximum toxin titers were obtained from control homogenates after 3 days incubation; the titer obtained from homogenates inoculated with the Tennessee strain was greater than that of the Saratoga strain. Microscopic examination of these homogenates at the 3-day sampling revealed the presence of refractile spores, vegetative cells, and lysed cells. Outgrowth and toxin production were

TABLE 2. Effects of NaCl concentration on outgrowth and toxin production of *Clostridium botulinum* type E (Saratoga or Tennessee strain) in cod homogenates during incubation at 30 C.

Brine %	Moisture %	pH	Incubation (*days*)	Outgrowth		Toxin (*MLD/g*)	
				S[a]	T[b]	S	T
0	89.4	6.9	3	+[c]	+	75	1000
			4			25	800
2.74	88.8	6.7	3	+	+	0	300
			6			4	25
3.84	87.6	6.6	4	–[d]	+	0	0
			6	+		0	16
			10	+		0	30
			30			0	*[e]
4.40	87.4	6.6	6	–	–	0	0
			10	+	+	0	*
			30			0	
5.50	86.2	6.6	10	–	–	0	0
			14	–	–		
			30	–	–	0	0

[a]Saratoga strain.
[b]Tennessee strain.
[c]Outgrowth.
[d]No outgrowth.
[e]Toxin present.

delayed or inhibited with increasing brine concentrations. The Saratoga strain was less salt-tolerant than the Tennessee strain. After 4 days incubation, direct counts (sporangia plus vegetative cells) of homogenates containing 2.74% brine were 40×10^6/g and 80×10^6/g for the Saratoga and Tennessee strains respectively. At the 3.84% brine level, outgrowth of the Saratoga strain was inhibited, whereas there was slight outgrowth of the Tennessee strain. Outgrowth of the two strains was inhibited in homogenates containing 5.0% added NaCl (5.50% brine); similar results concerning the inhibitory effect of 5.0% NaCl concentration on growth of type E strains in two similar trypticase–peptone media have been reported (Segner et al. 1966; Emodi and Lechowich 1969). Outgrowth and toxin production were obtained in subcultures of the Saratoga and Tennessee strains from the 5.50% brine samples, indicating that NaCl at this concentration exerted a sporistatic effect.

A brine concentration of 3.84% inhibited toxin formation in homogenates inoculated with the Saratoga strain. This is in agreement with results presented by Bohrer et al. (1965).

The Tennessee strain was more tolerant to NaCl in respect to toxin production than either the Minnesota or Saratoga strain. A brine concentration of 4.40% (4.0% NaCl) suppressed toxin production, but toxin of low potency was produced after 10 days incubation. Abrahamsson et al. (1966) reported that toxin was formed by type E strains during growth in Robertson's chopped meat medium containing 4.0% added NaCl.

During preliminary studies concerning the NaCl tolerance of type E strains, smoked Pacific cod homogenates containing various levels of added NaCl were used as substrates. The results of these tests revealed that a brine concentration of 3.90% inhibited toxin formation, but did not inhibit growth (J. W. Boyd and B. A. Southcott 'unpublished data). Kautter (1964) reported that vacuum-packaged smoked ciscoes containing 3.3% NaCl (4.76–4.82% brine) supported growth of *C. botulinum* type E, and became toxic. Sheneman (1965) found that smoked whitefish chubs with a brine content of 5.50% were unable to support toxin production; toxin was formed in fish with a brine level of 3.0%. A brine concentration greater than 3.0% in smoked chubs inhibited toxin production (Christiansen et al. 1968).

The results as presented in this paper were obtained from tests carried out at 30 C, the optimum temperature of growth for type E strains. The NaCl tolerance of type E strains is reduced at lower temperatures of incubation. During incubation of inoculated fresh fish homogenates (*p*H 6.5) at 7 C for 1 year, Bohrer et al. (1965) found that a brine concentration of 3.17% supported growth of type E strains; 3.94% inhibited growth. At 29.4 C incubation, growth was not inhibited at the 3.94% brine level. Segner et al. (1966) reported that a 4.0% NaCl concentration in TPG medium had no major effect on delay in outgrowth time at 30 C; at 8 C it produced a three- to fourfold extension. Emodi and Lechowich (1969) found that during incubation at 5.0 and 15.6 C the salt tolerance of type E strains was 2.5 and 4.5% NaCl respectively.

The present study has shown that there is a slight variation in the salt tolerance between certain type E strains. At the optimum temperature of growth, a minimum brine concentration of 5.50% (5.0% NaCl w/w) would be required in certain substrates to inhibit toxin production.

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Some Physical Oceanographic Features of Inlets of Chile

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PICKARD, G. L. 1971. Some physical oceanographic features of inlets of Chile. J. Fish. Res. Bd. Canada 28: 1077–1106.

The first systematic study of physical oceanographic characteristics of the fjord inlets of Chile was carried out by the Institute of Oceanography of the University of British Columbia during March 1970 as Phase V of the *Hudson* 70 Round-the-Americas Expedition of the Bedford Institute, Canada Department of Energy, Mines and Resources.

Observations were made of temperature, salinity, and dissolved oxygen at 175 stations in 32 inlets and in the passages outside them, and soundings were made along 3500 km of track. The sounding data are presented as longitudinal sections, and water properties are summarized in temperature–salinity and temperature–dissolved oxygen correlation plots and characteristic diagrams; comparisons are made with observations for the British Columbia and Alaska inlets reported by Pickard in 1961 (18: 907–999) and 1967 (24: 1475–1506) in the Journal of the Fisheries Research Board of Canada. Observations were also made of nutrients in some Chilean inlets; these will be reported at a later date.

In mean and extreme values, the dimensions of the inlets in Chile are similar to those in British Columbia/Alaska; only two shallow sills were recorded. More inlets had glaciers contributing melt water directly to them and more had icebergs present than is the case in the northeast Pacific inlets. Vertical salinity profiles in Chile were similar to those in British Columbia/Alaska, but the thinner surface layer was of higher salinity, except in the inlets having icebergs, and the deepwater salinities were 1–2‰ higher in Chile. Temperatures were 2–5 degrees C higher in Chile except in the iceberg inlets where surface values were lower than those in Alaska. Several of the iceberg inlets in Chile had much more complicated temperature/depth profiles than in Alaska, with as many as seven maxima and minima below the surface. The glacial silt in the inlet waters was more conspicuous and extensive in Chile than in British Columbia/Alaska. Dissolved oxygen values in Chile were much the same as in British Columbia/Alaska with higher values in the iceberg inlets in Chile than in Alaska, and there was little evidence of very low values characteristic of stagnant basins. The lack of low dissolved oxygen values suggests free exchange with outside waters and the inlet deepwater characteristics indicate that the effective sill depths between inlets and the ocean are only 100–150 m deep. At least four distinct major regions are evident from water properties among the inlets of Chile.

PICKARD, G. L. 1971. Some physical oceanographic features of inlets of Chile. J. Fish. Res. Bd. Canada 28: 1077–1106.

La première étude systématique de l'océanographie physique des fjords du Chili fut accomplie par l'Institut d'Océanographie de l'Université de Colombie Britannique en mars 1970. Ce projet était la cinquième phase de l'expédition *Hudson* 70 autour des Amériques organisée par l'Institut Bedford du Ministère de l'Energie, des Mines et des Ressources du Canada.

Nous avons mesuré la température, la salinité et l'oxygène dissous à 175 stations dans 32 fjords et dans les passages extérieurs, et nous avons fait 3500 km. de sondages. Les sondages sont présentés sous forme de diagrammes de température–salinité et de température–oxygène. Ces observations sont comparées à celles faites dans les fjords de Colombie Britannique et d'Alaska, déjà publiées par Pickard en 1961 (18: 907–999) et 1967 (24: 1475–1506) dans le *Journal of the Fisheries Research Board of Canada*. Des mesures des concentrations de sels nutritifs dans quelques-uns des fjords du Chili seront publiées plus tard.

Les dimensions des fjords du Chili ressemblent à celles des fjords de Colombie Britannique et d'Alaska; seulement deux de ces fjords ont un seuil peu profond. On trouve toutefois au Chili plus de fjords où des glaciers contribuent directement de l'eau de fonte et des icebergs qu'en Colombie Britannique/Alaska. Les profils verticaux de salinité de l'eau des fjords chiliens ressemblent à ceux de Colombie Britannique/Alaska, mais la couche de surface y est plus

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salée (sauf où flottent des icebergs) et la salinité des eaux profondes est plus haute au Chili de 1–2‰. Les eaux au Chili sont plus chaudes de 2–5 C. qu'en Colombie Britannique/ Alaska, sauf dans les fjords à icebergs où les eaux de surface sont plus froides. Plusieurs des fjords à icebergs du Chili ont des profils verticaux de température plus complexes qu'en Alaska, avec jusqu'à sept maxima et minima sous la surface. La vase des glaciers ("poudre de roche") dans les eaux des fjords est plus évidente et plus répandue au Chili qu'en Colombie Britannique/ Alaska. La concentration en oxygène dissous est à peu près la même au Chili, mais dans les fjords à icebergs du Chili les concentrations sont plus élevées, et on ne trouve pas les concentrations très basses qui caractérisent les bassins stagnants. L'absence de basses concentrations d'oxygène suggère que l'eau des fjords se mêle sans encontres aux eaux des passages extérieurs; les caractéristiques des eaux des fjords indiquent de plus que les seuils qui les séparent de l'océan n'ont que de 100–150 m. de profondeur. On distingue, d'après les caractéristiques générales de ces eaux, quatre régions océanographiques principales le long de la partie sud de la côte du Chili, découpée de fjords.

Received March 31, 1971

THE southern thousand miles of the coast of Chile contains one of the major fjord regions of the world, but until the present work in 1970 there had been no systematic oceanographic study of the waters of this region.

Members of the Institute of Oceanography of the University of British Columbia had studied the inlets of British Columbia and Alaska on the west coast of North America for many years (Pickard 1961, 1963, 1967) and had for some time desired to study those on the west coast of South America. The opportunity to do so arose as a part of the Round-the-Americas expedition by the C.S.S. *Hudson* (*Hudson* 70 Expedition by the Bedford Institute, Marine Sciences Branch of the Canada Department of Energy, Mines and Resources). The study of the Chilean fjord inlets was included as Phase V of that cruise, from March 4 to 30, 1970. The Institute of Oceanography designation is the "Chile 70" cruise.

The object of the study was to learn as much as possible about the oceanography of the region to add to the available knowledge of fjord regions and to compare with other fjord regions, particularly that of the northeast Pacific (British Columbia and Alaska). As the time available was limited, it was decided to observe the distribution of water characteristics in as many fjords as possible distributed along the entire coast, taking stations at intervals of 8–16 km (5–10 nautical miles). In the absence of any previous work, the selection was somewhat arbitrary but with emphasis on the longer fjords. Because of our interest in estuarine regions, preference was given to fjords in the mainland coast that might be expected to experience significant river runoff, rather than to those in the large islands separated from the mainland by the narrow waterways that form an "inland passage" along the coast.

One of the difficulties in planning the Chile 70 cruise was that although the inland passage was well enough surveyed to allow the passage of deep-sea vessels, many of the fjords were not surveyed at all. The intention was to have the sounding launches precede the ship in the unsurveyed waters. Unfortunately, equipment problems made it impractical to use the launches and it was considered imprudent to work with the ship itself in the unsurveyed areas. Data were obtained in some of these from a ship's lifeboat, using a hand winch, but it was not possible to transfer the echo sounders from the launches to the lifeboat and therefore soundings were not obtained in the regions studied from the lifeboat.

The primary purpose of this paper is to present a broad summary of the observed oceanographic characteristics of the Chilean fjords, as these have not previously been described. Some comparisons with the fjord characteristics of North America are also presented, but discussion of many of the details of the features observed in Chile is left until later.

Water samples were obtained using Copenhagen reversing water bottles and temperatures were measured with Richter and Wiese reversing thermometers and temperature profiles with a 270 m bathythermograph. The salinity was determined with an AutoLab Inductive Salinometer (range 0–40‰) and the dissolved oxygen content by a modified Winkler titration, using reagents as recommended by Carritt and Carpenter (1966). (Nutrients were determined at some stations using a Technicon AutoAnalyser — these results will be reported later by I. W. Duedall.) In addition, temperature and salinity profiles were obtained with a Bissett-Berman model 9060 Selfcontained Salinity, Temperature, Depth (STD) Recorder (0–500 m depth range) using the 30–40‰ or 0–40‰ salinity range as appropriate.

In the inlets and inside passages, 175 stations were occupied for reversing water-bottle casts and 148 STD and 150 bathythermograph casts were made; in addition four stations were occupied for bottle, STD, and bathythermograph casts at stations in the Pacific Ocean off entrances to the inside passages. Meteorological observations were made at each station and Secchi disc measurements made at stations occupied in daylight.

The Chilean charts were used for navigation, and positions were determined by radar fixes on shore marks. Continuous echo soundings were made throughout the cruise.

General Characteristics of the Region

Topography

Figure 1 shows on the right the location of the fjord inlet region of Chile on the Pacific coast of South America, and on the left is shown the Pacific coast of North America rotated through 180° to facilitate comparison with the southern fjord coast in terms of latitude. Figure 2 shows the Chilean region on a larger scale, with the studied inlets named and locations given for other oceanographic stations and for climatological stations referred to later.

The fjord inlet region (Fig. 2) extends from near Puerto Montt at 41.4°S to Tierra del Fuego at about 55°S, south of the Estrecho de Magallanes. The region is generally rugged, with relief of 500–2000 m in the immediate neighbourhood of the inlets, the central part of the area having the greatest relief. The mainland coast is separated from the Pacific Ocean by a large number of islands and narrow passages, the majority of these being unsounded.

In the northern third of the region, from about 41.4°S to 46.5°S, most of the mountains close to the inlets are covered with trees to 1500 m elevation or more, but south of 47°S the tree line descends until in the Estrecho de Magallanes region it is at some 500 m or less above sea level. Very conspicuous features, both visually and in their effect on the coastal waters, are the many glaciers south of 46°S. Some descend from large ice fields in the mountains to sea level in the inlets; others are just small caps on rounded mountains at elevations of 500–1000 m. The areas of light-coloured rock surrounding the small cap glaciers suggest that the latter are decreasing in size. In several of the inlets ice was encountered during this cruise with bergy bits up to about 5 m in length. These will be referred to as "iceberg inlets."

Longitudinal profiles of the inlets traversed by the C.S.S. *Hudson* (Fig. 3 and 4) were prepared from the continuous sounding records made during the Chile 70 cruise and from the Chilean charts. These profiles are only first approximations because the midchannel course followed by the ship does not necessarily follow the line of maximum depth. In addition, there were some places where the soundings obtained during the runs in and out of an inlet were significantly different. Such differences could be due to the irregular character of the bottom in many areas and to the fact that navigation by

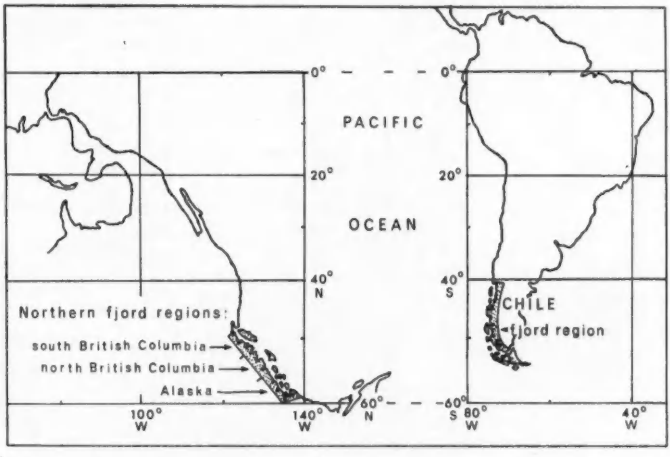

FIG. 1. Right, location of fjord inlet region of Chile; left, location of fjord inlet region of British Columbia/Alaska rotated 180° to show relative latitudes.

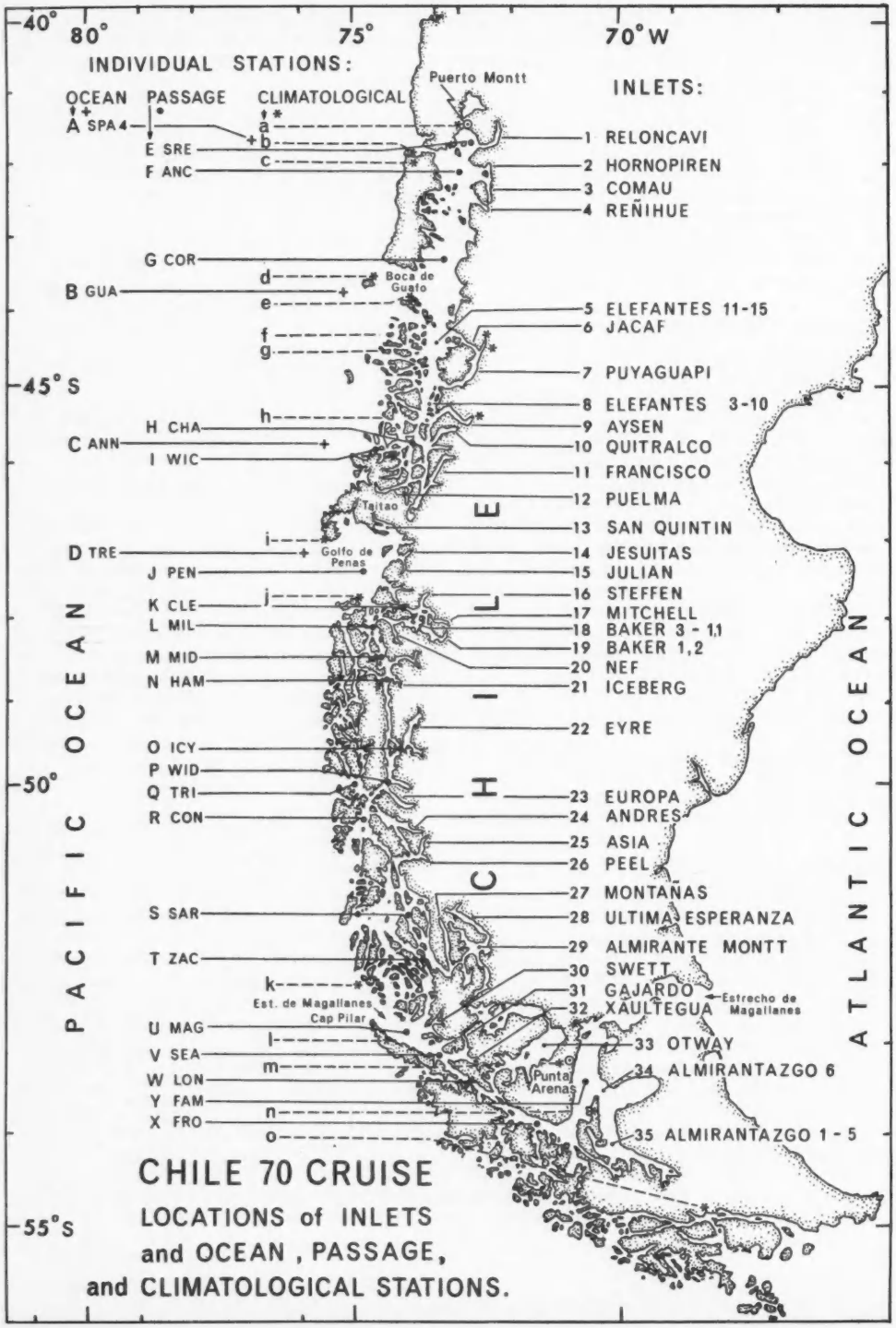
40°
80°
75°
70°W
INDIVIDUAL STATIONS:
OCEAN
PASSAGE
CLIMATOLOGICAL
A SPA 4
E SRE
F ANC
G COR
B GUA
H CHA
C ANN
I WIC
D TRE
J PEN
K CLE
L MIL
M MID
N HAM
O ICY
P WID
Q TRI
R CON
S SAR
T ZAC
U MAG
V SEA
W LON
Y FAM
X FRO
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
Puerto Montt
Boca de Guafo
Taitao
Golfo de Penas
Est. de Magallanes
Cap Pilar
Punta Arenas
Estrecho de Magallanes
INLETS:
1 RELONCAVI
2 HORNOPIREN
3 COMAU
4 REÑIHUE
5 ELEFANTES 11-15
6 JACAF
7 PUYAGUAPI
8 ELEFANTES 3-10
9 AYSEN
10 QUITRALCO
11 FRANCISCO
12 PUELMA
13 SAN QUINTIN
14 JESUITAS
15 JULIAN
16 STEFFEN
17 MITCHELL
18 BAKER 3 - 11
19 BAKER 1,2
20 NEF
21 ICEBERG
22 EYRE
23 EUROPA
24 ANDRES
25 ASIA
26 PEEL
27 MONTAÑAS
28 ULTIMA ESPERANZA
29 ALMIRANTE MONTT
30 SWETT
31 GAJARDO
32 XAULTEGUA
33 OTWAY
34 ALMIRANTAZGO 6
35 ALMIRANTAZGO 1 - 5
45°S
50°
55°S
PACIFIC OCEAN
ATLANTIC OCEAN
CHILE
CHILE 70 CRUISE
LOCATIONS of INLETS
and OCEAN , PASSAGE,
and CLIMATOLOGICAL STATIONS.

FIG. 2 Locations of inlets and of ocean and passage stations studied in Chile 70 cruise, and of climatological stations used for reference. (See Tables 1–3 for keys to station-identifying letters.)

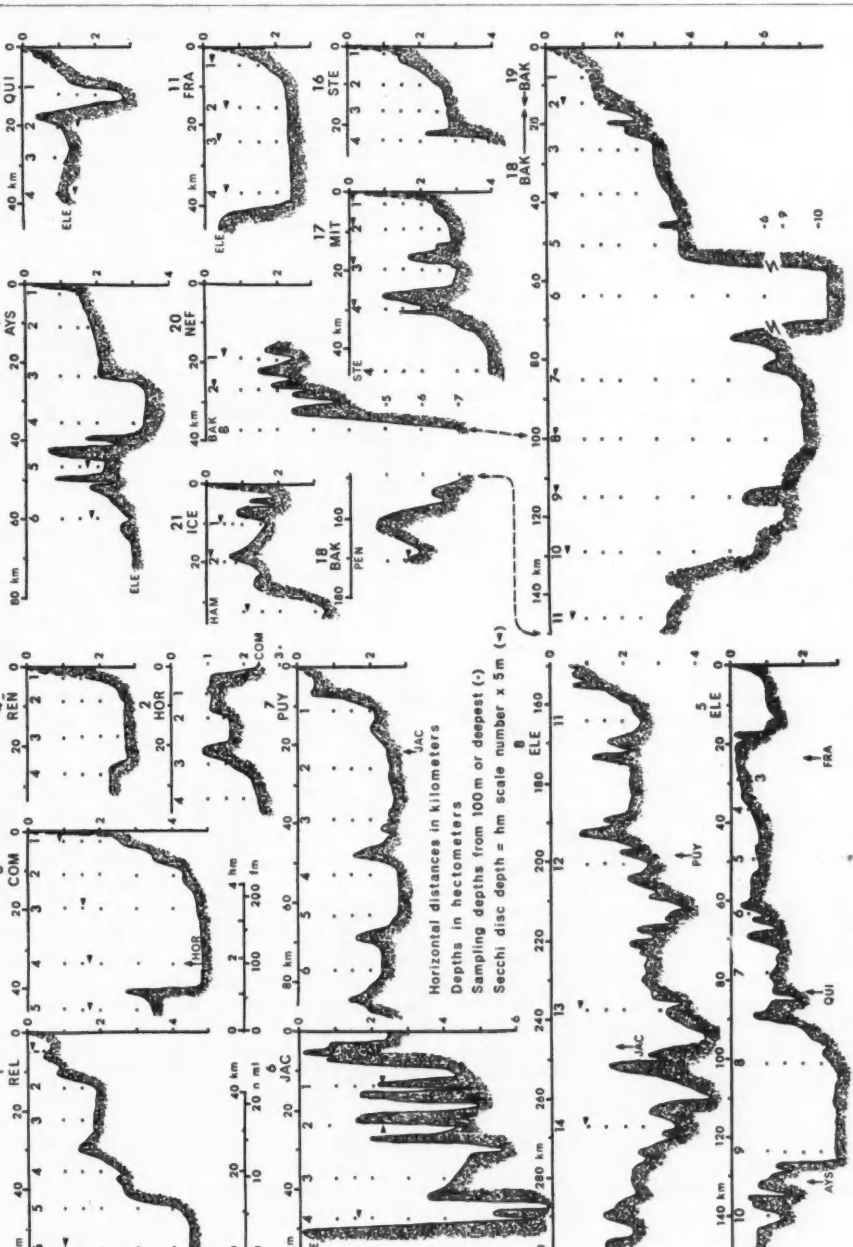

FIG. 3. Longitudinal sections along inlets studied in Chile 70 cruise, numbers 1–21 in northern part of coast.

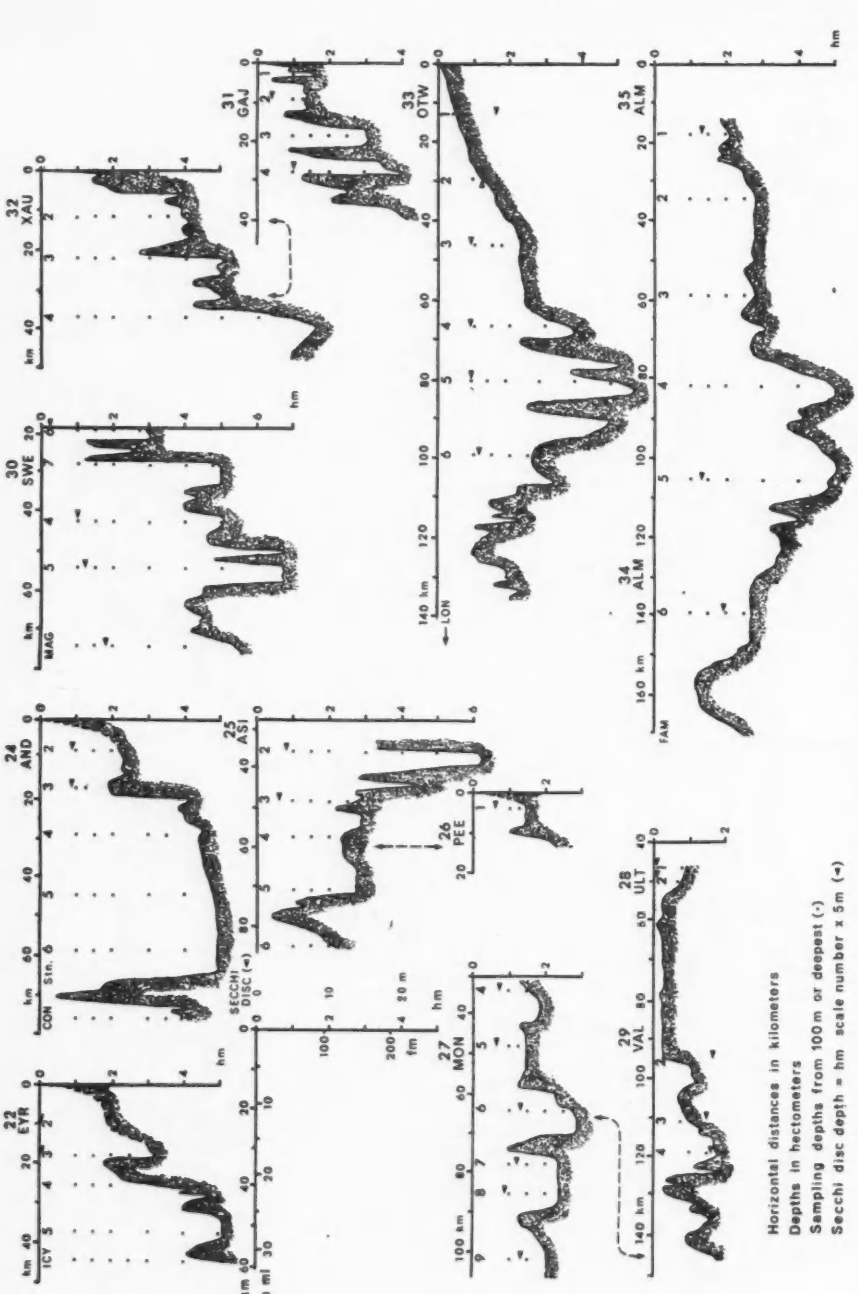

FIG. 4. Longitudinal sections along inlets studied in Chile 70 cruise, numbers 22–35 in southern part of coast.

radar fixes on the shoreline is not accurate enough for good sounding runs over the prevailing rugged bottom topography. In preparing the longitudinal sections, shallow soundings (suggesting sills) that appeared on the ship's records have been ignored unless supported by soundings on the Chilean charts and by significant differences in the water characteristics below the apparent sill depth on either side. No attempt has been made to correct for tidal height in preparing the profiles. The range of spring tides for most of the coast is only about 2 m, with higher values up to about 6 m only in the Golfo Reloncavi area near Puerto Montt in the north.

In Table 1 are given the names and dimensions of the inlets studied; the depths have been rounded off to the nearest 5 m because of the limitations of the data. The "outer sill depth" is that of the shallowest sill in the outer one-third of the inlet length. It should be noted that these sills are between the inlets and the inside passages or straits; none of the inlets studied opens directly to the Pacific Ocean. Generally there are depths between the inside passages and the ocean that are shallower than much of the passages themselves. The deepest connections to the Pacific are probably through the Boca del Guafo (43.5°S) and Golfo de Penas (47.5°S) with depths of about 150 m; the connections further south appear from the charts to have least depths of less than 100 m.

As in the northeast Pacific fjord region, several designations are used for the bodies of water without any apparent oceanographic consistency. The most common are "canal" (= canal, channel, passage), "estero" (= bay, cove, channel, estuary, strait), "estuario" (= estuary), and "seno" (= bay). For convenience, the bodies of water studied will be referred to in general as inlets (because not all of them are fjords), and the names mentioned above (e.g., canal, estero, etc.) will be omitted when referring to specific inlets.

In Table 1 and Fig. 2 the inlets are listed from north to south by name and also by an identifying number starting with 1 at the north. On the figures showing water properties, an inlet will generally be identified by its number as this is more compact and will more readily indicate to most readers the inlet's position along the coast than would its proper name. Two identifying numbers are used for three inlets (Elefantes 5 and 8, Baker 18 and 19, and Almirantazgo 34 and 35) as the water properties showed significant differences between sections of these inlets, usually due to the presence of a sill. Station positions along each inlet are numbered starting with 1 at the inland end. The stations in the Pacific off the fjord coast and those in the passages are identified by letters in Fig. 2 and in diagrams of water properties. The positions of these stations are given in Table 2.

Anticipating the oceanographic results to be presented, it is convenient to divide the region into three main areas, the northern from Puerto Montt (41.4°S) to the Peninsula de Taitao (46.5°S), the middle from there to the Cap Pilar (western) entrance to the Estrecho de Magallanes (about 52.7°S), and the southern from there to Seno Almirantazgo (54.5°S). In the middle region, the water characteristics suggest a further subdivision into the north middle (about 47–48.5°S around Canal Baker, 18 and 19), the iceberg inlets (48.5–51°S), and the south middle.

Climate

The southern part of Chile is very sparsely populated and there are not many climatological stations along the inlet coast (see Fig. 2 where climatological stations are indicated by lower case letters). In particular there is a big gap between 48° and 52.5°S. However, a general summary of the climate has been assembled from the paper by Reed (1929), from tables in the USNOO Sailing Directions (Anon. 1952), and from data supplied by Sr H. Sievers, Head, Centro Nacional de Datos Oceanograficos, Chile. The stations used are listed in Table 3 and the locations shown on Fig. 2.

The monthly mean air temperature has a maximum in January or February and a minimum in July or August (Fig. 5). The annual mean air temperature decreases roughly linearly from 10 C in the north to 5 C in the south (Fig. 6), with an annual range of monthly mean temperature of 6 C in the north to 9 C in the south.

The rainfall from 42°S to 53°S averages about 2700 mm per year, with a maximum of 5090 mm per year at the Pacific entrance to the Estfecho de Magallanes (Fig. 6). These data are for the outer coast — the values for the inlets themselves among the coastal mountains could be higher. An indication of the local variability is given by comparing the annual rainfalls at two pairs of stations: Guafo (*d*) with 1040 mm and Melinka (*e*) with 3170 mm, which are 80 km apart, and Rio Cisnes (*g*) with 590 mm and Puerto Puyuhuapi (*f*) with 3870 mm, which are only 20 km apart. The rainfall at the southern and more easterly stations of Punta Arenas (*m*), Bahia Harris (*n*), and Ushuaia (*o*) is considerably less at about 600 mm but these stations are in the rain shadow of the mountains to their west.

The rainfall has a maximum in winter (May–August) in the northern area (e.g., Fig. 5, station *c*)

TABLE 1. Salient dimensions of inlets studied in Chile 70 cruise. Depths taken from Chilean charts or soundings, or both, by C.S.S. *Hudson*, all rounded to nearest 5 m, no correction for tide height.

No.	Abbrev-iation	Name	Length (*km*)	Mean width (*km*)	Mean midinlet depth (*m*)	Max depth (*m*)	Outer sill depth (*m*)	Glacier[a]	Ice in water[b]	Notes
1	REL	Estero Reloncavi	56	2.7	225	450	None			
2	HOR	Canal Hornopiren	37	2.2	160	250	None	V. small		
3	COM	Estero Comau	41	4.5	420	480	270			
4	REN	Estero Reñihue	28	5.1	245	280	230			
5,8	ELE	Estero Elefantes	300	11.5	185	440	150			c
6	JAC	Canal Jacaf	52	3.8	340	690	25			
7	PUY	Canal Puyuguapi	100	4.2	220	280	140	Possible		
9	AYS	Seno Aysen	70	4.2	225	335	70			
10	QUI	Estero Quitralco	39	3.4	125	275	100			
11	FRA	Estuario Francisco	44	2.7	200	240	40			
12	PUE	Estero Puelma	89	2.0	...	>150	...			d
13	SQU	Bahia San Quintin	32	8.2	30	60	None			
14	JES	Seno Jesuitas	19	2.2	...	>200	...	Yes		d
15	JUL	Estero Julian	22	0.8	...	>200	...	?		d
16	STE	Estero Steffen	24	3.4	240	285	220	Yes		
17	MIT	Estero Mitchell	45	2.3	265	390	140			
18,19	BAK	Canal Baker	160	3.1	470	1050	75	Yes	Some	e
20	NEF	Estero Nef	37	1.3	275	370	240			f
21	ICE	Seno Iceberg	26	1.9	125	160	70	Yes	Yes	
22	EYR	Seno Eyre	46	4.6	310	510	410	Yes	Yes	
23	EUR	Seno Europa	56	3.0	...	>200	...	Yes	Yes	d
24	AND	Canal & Estero Andres	70	4.4	365	500	50			
25	ASI	Estero Asia	85	3.2	265	620	45	Yes	Yes	g
26	PEE	Estero Peel	9	2.9	140	150	105	Yes	Yes	h
27	MON	Estero de las Montañas	102	3.3	225	290	100	Yes		i
28	ULT	E. Ultima Esperanza	56	...	...	>80	10	Possible		d
29	VAL	Golfo Almirante Montt	35	5.9	75	180	30			j
30	SWE	Canal Swett	59	2.8	445	670	410	Yes		k
31	GAJ	Canal Gajardo	41	1.7	200	400	130	Yes		
32	XAU	Golfo Xaultegua	46	4.6	465	760	430	Small		
33	OTW	Seno Otway	124	15.0	215	540	90			
34,35	ALM	Seno Almirantazgo	163	13.7	300	500	110	Yes		l
Mean values etc:										
Chile		32 inlets	66	6.1	265	410	145	14	6	
British Columbia		44 "	47	2.3	310	385	125	6	None	
Alaska		16 "	117	9.5	350	480	155	8	4	

[a]From Anon. (1968) and observation during cruise.
[b]Observed in March 1970.
[c]Inlet 5 is Canal Moraleda, stations ELE 11–15, and inlet 8 is Estero Elefantes, stations ELE 3–10, the former being geographically a continuation of the latter.
[d]Insufficient soundings available.
[e]Inlet 19 is Estero Steele, stations BAK 1 and 2, and inlet 18 is Canal and Seno Baker, stations BAK 3–11, the latter being a continuation of the former.
[f]Nef — soundings for outer 22 km.
[g]Comprising Estero Asia and outer part of Estero Peel; soundings for outer 50 km.
[h]Side arm from junction of Asia and outer part of Peel.
[i]Comprising Seno Union and Canal de las Montañas, soundings for outer 60 km.
[j]Comprising Canal Valdes and Golfo Almirante Montt.
[k]Comprising Bahia Beaufort, Seno Glacier, and Canal Swett; soundings for outer 40 km.
[l]Inlet 34 station ALM 6, inlet 35 is stations ALM 1–5, the two forming Seno Almirantazgo.

TABLE 2. Ocean and passage station positions, Chile 70 cruise.

Identifying letter	Abbreviation	Location	Lat S	Long W	Depth (*m*)
		Ocean stations			
A	SPA4	W of Canal Chacao	41°40.0′	77°00.0′	2950
B	GUA	W of Isla Guafo	43 45.5	75 11.7	990
C	ANN	W of Bahia Anna Pink	45 45.0	75 40.2	970
D	TRE	W of Cabo Tres Montes	47 00.4	75 47.5	1960
		Passage stations			
E	SRE	Seno Reloncavi	41 43.1	72 45.2	350
F	ANC	Golfo de Ancud	42 04.2	73 09.0	340
G	COR	Golfo Corcovado	43 18.4	73 17.6	225
H	CHA	Canal Chacabuco	45 44.6	73 59.2	135
I	WIC	Boca Wickham	45 00.0	74 30.6	240
J	PEN	Golfo de Penas	47 34.5	74 54.1	190
K	CLE	Canal Martinez	47 47.8	74 16.6	275
L	MIL	Canal Messier	48 00.8	74 41.4	1000
M	MID	" "	48 25.6	74 32.6	1280
N	HAM	" "	48 32.0	74 26.7	350
O	ICY	Canal Icy	49 38.5	74 14.1	500
P	WID	Canal Wide	49 59.4	74 26.2	1080
Q	TRI	Canal Trinidad	50 01.7	74 55.2	300
R	CON	Canal Concepcion	50 20.4	74 49.5	405
S	SAR	Canal Sarmiento	51 36.6	73 59.8	930
T	ZAC	Canal Smyth	52 01.5	73 46.2	380
U	MAG	Estrecho de Magallanes (W)	52 54.1	73 56.1	550
V	SEA	Paso Largo	53 07.8	73 25.0	1130
W	LON	Paso Largo	53 20.8	73 02.2	820
X	FRO	Paso Froward	53 51.0	71 38.0	300
Y	FAM	Estrecho de Magallanes (E)	53 29.2	70 37.2	410

TABLE 3. Climatological stations in southern Chile: positions and sources of data.

Identifying letter	Station name	Lat S	Long W	Sources of data[a]
a	Puerto Montt	41°21′	72°56′	CNDOC
b	Puerto Corona	41 47	73 52	CNDOC
c	Ancud	41 52	73 49	CNDOC
d	Isla Guafo	43 34	74 45	USNOO
e	Melinka	43 54	73 45	Reed
f	Puerto Puyuhuapi	44 20	72 28	CNDOC
g	Rio Cisnes	44 32	72 24	CNDOC
h	Puerto Aysen	45 24	72 42	CNDOC
i	Cabo Raper	46 50	75 35	CNDOC
j	San Pedro	47 43	74 55	CNDOC
k	Los Evangelistas	52 24	75 06	USNOO
l	Bahia Felix	52 58	74 05	Reed
m	Punta Arenas	53 10	70 54	USNOO
n	Bahia Harris	53 50	70 24	Reed
o	Ushuaia	54 50	68 20	USNOO

[a]Sources: CNDOC, Centro Nacional de Datos Oceanograficos, Chile; USNOO, Anon. (1952); Reed, 1929 paper.

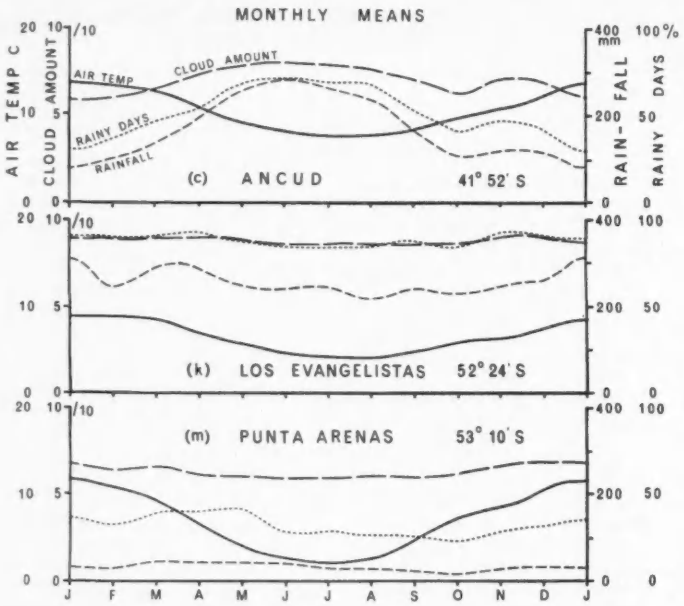

FIG. 5. Annual variation of monthly mean air temperature, rainfall, rainy day frequency, and cloud amount at selected stations in fjord inlet coast of Chile.

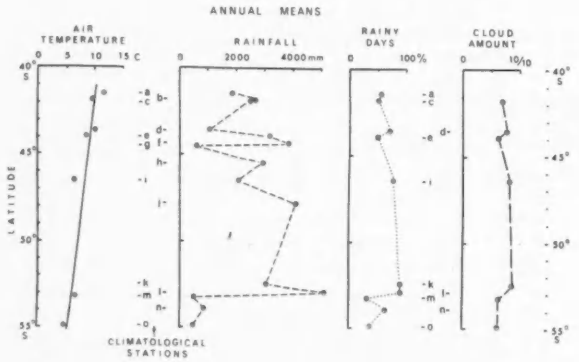

FIG. 6. Variation with latitude of annual mean air temperature, rainfall, rainy day frequency, and cloud amount for fjord inlet coast of Chile. (See Table 3 for station key.)

but south of about 47°S it is fairly evenly distributed through the year (e.g., Fig. 5, station *k*). In the middle part of the coast there is rain on 80–90% of days of the year, in the north and south on 30–50% of days (Fig. 6). The cloud cover averages $\frac{8}{10}$–$\frac{9}{10}$ in the middle coast and $\frac{6}{10}$–$\frac{7}{10}$ elsewhere (Fig. 6).

The general impression from accounts of the climate of the fjord region is of an inhospitable region typified by a paragraph in the USNOO Sailing Directions referring to one of the channels: "The navigation is perfectly easy in moderately fine weather: the real difficulty of it consists in the

almost perpetual rain, thick weather, and strong gales, with very heavy williwaws, which come down the steep mountain sides with a force which must be felt to be believed." If this is a correct assessment, we were fortunate in having better than average weather during the Chile 70 cruise, with rain on only 40% of the days, mean cloud cover of 5.5/10, and frequency of wind speeds as follows: calm or light airs 34%; 1–10 mph 23%; 11–20 mph 29%; 21–30 mph 8%; 31–60 mph 6%.

Results

Temperature, Salinity, and Oxygen

The values for water temperature, salinity, and dissolved oxygen are presented in this paper in graphical form rather than in numerical tabulation. In diagrams of publishable size the former method is less precise than the latter but is more compact and better adapted for displaying comparisons within the area of study and with regions elsewhere. The lower precision is considered not significant in view of the probability that seasonal and year-to-year variations in water properties may be expected to be considerably larger than the precision of measurement by the techniques used. The measured values are tabulated in a data report (Anon. MS 1971) of the Institute of Oceanography, University of British Columbia. The data are also deposited with the Centro Nacional de Datos Oceanograficos de Chile, Cascilla 324, Valparaiso, Chile and with the Instituto Central de Fisica, University of Concepción, Chile.

Temperature–Salinity and Temperature–Oxygen Correlations

It was found that although there were significant horizontal variations in water properties, particularly salinity, in some inlets in the upper 20 m of water, the major systematic variations were in the vertical direction in the upper 200 m. For the first description, therefore, water characteristics are displayed as temperature–salinity (T–S) and temperature–oxygen ($T–O_2$) correlation plots for selected depths of 0, 20, 50, 100, and 200 m in Fig. 7–11. Some information on water properties at greater depths is given later. In reducing the data for presentation on these plots, the mean value of the water property at each depth in each inlet was calculated and the range of values also noted (maximum minus minimum value). On the figures, the mean value of temperature–salinity or temperature–oxygen for each inlet is indicated by the number of the inlet as given in Table 1. To indicate the significance of the separation of the points, a "reduced mean range" of properties is shown on each figure. This range was obtained by first calculating the mean of the ranges for all inlets at that depth, then extracting those values more than three times the first mean, and calculating the mean of the remainder ("reduced mean"). The reduced mean range and the extracted values are given on each figure. Note that the reduced mean ranges are generally small compared with the differences in mean values between inlets, and that geographic variations of mean properties are evident. The northern inlets (1–12) are in the short-dash envelope, the north middle ones (14–20) in the dot-dash envelope, the southern group (30–35) are within the full-line envelope, and the inlets with glacier ice in the water are indicated by triangles and connected by a long-dash line.

In Fig. 7–11 are also shown values for T–S and $T–O_2$ correlations for one of the ocean stations (Guafo, B) and for the selection of passage stations for which T–S and $T–O_2$ diagrams are given in Fig. 14.

Salinity–Depth Profiles

In all the inlets visited, the surface salinity at the inlet head was less than at the mouth and, with a few minor exceptions mentioned in the next paragraph, the salinity increased with depth. Both of these are characteristics of estuarine regions. Most of the salinity increase occurred in the upper 20 or 30 m; in some instances there was an isohaline layer of a few meters depth from the surface. Table 4 summarizes some of the characteristics, i.e., the surface and deep salinity, the salinity–depth profile type in the upper layer (Fig. 12), the isohaline layer depth and the depths at which the salinity reached 50 and 90% of the deepwater salinity (to indicate the distribution of fresh water with depth).

The locations where there were decreases of salinity with increasing depth were at one station each in Comau (3) and Reñihue (4) in the north, and at four in Almirantazgo (35) in the south. The change of salinity was only 0.01–0.07‰, in all instances in the vicinity of 100 m depth. The STD traces confirmed these minor localized salinity inversions, which were compensated by temperature changes so that the water remained stable. Larger inversions of 0.03–0.12‰ were noted at three passage stations (F, I, and R) between 100 and 200 m, whereas the ocean stations showed a salinity maximum at 200–300 m with a decrease of 0.01–0.11‰ to a minimum at 500–600 m.

About 20% of the inlet stations showed a surface salinity higher than that at the first subsurface depth sampled (usually 2 or 5 m). During the early part of the cruise this phenomenon was correlated

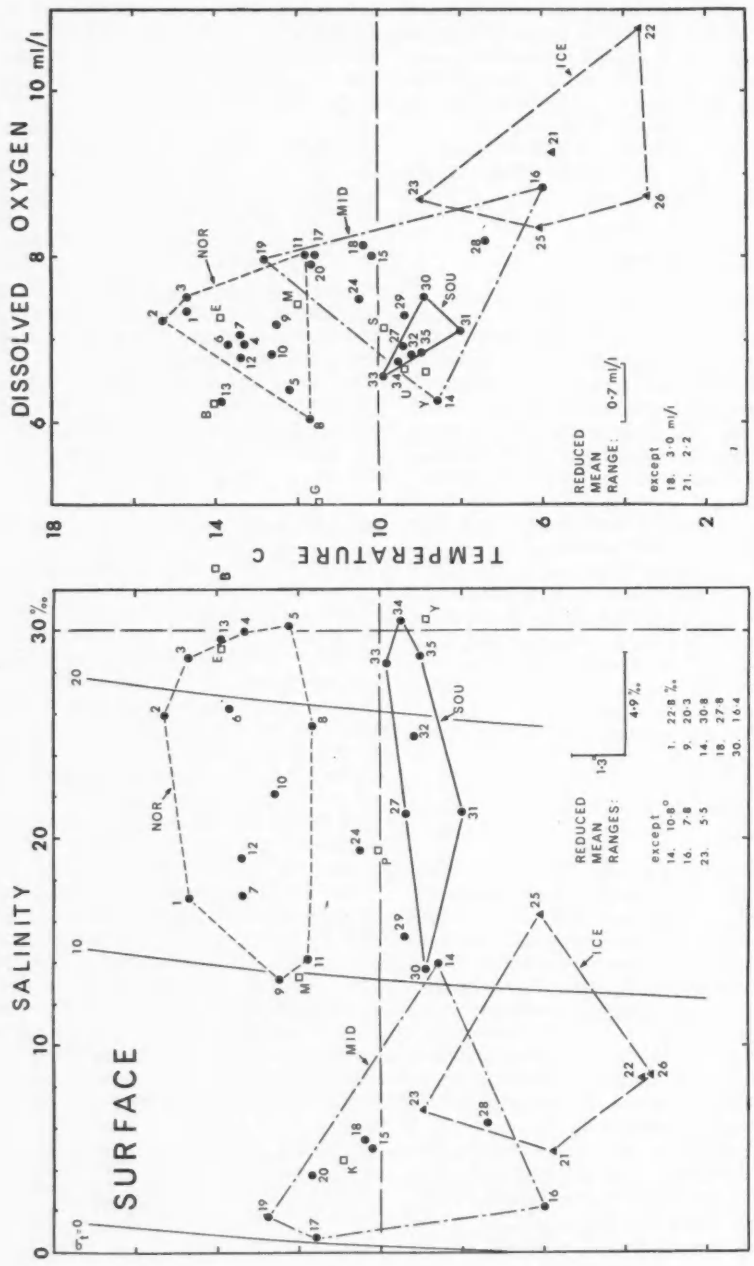

FIG. 7. Temperature–salinity and temperature–dissolved oxygen correlation plots for Chile inlets, Chile 70 cruise, surface values.

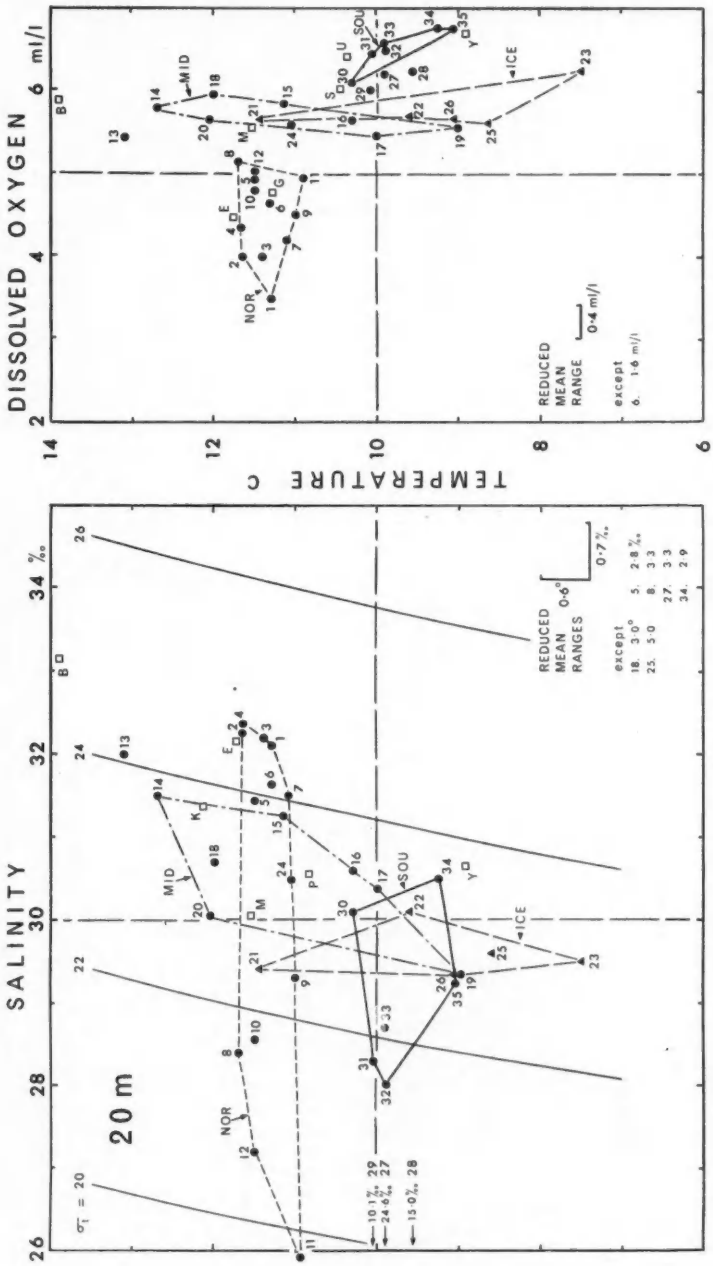

FIG. 8. Temperature–salinity and temperature–dissolved oxygen correlation plots for Chile inlets, Chile 70 cruise, 20 m depth.

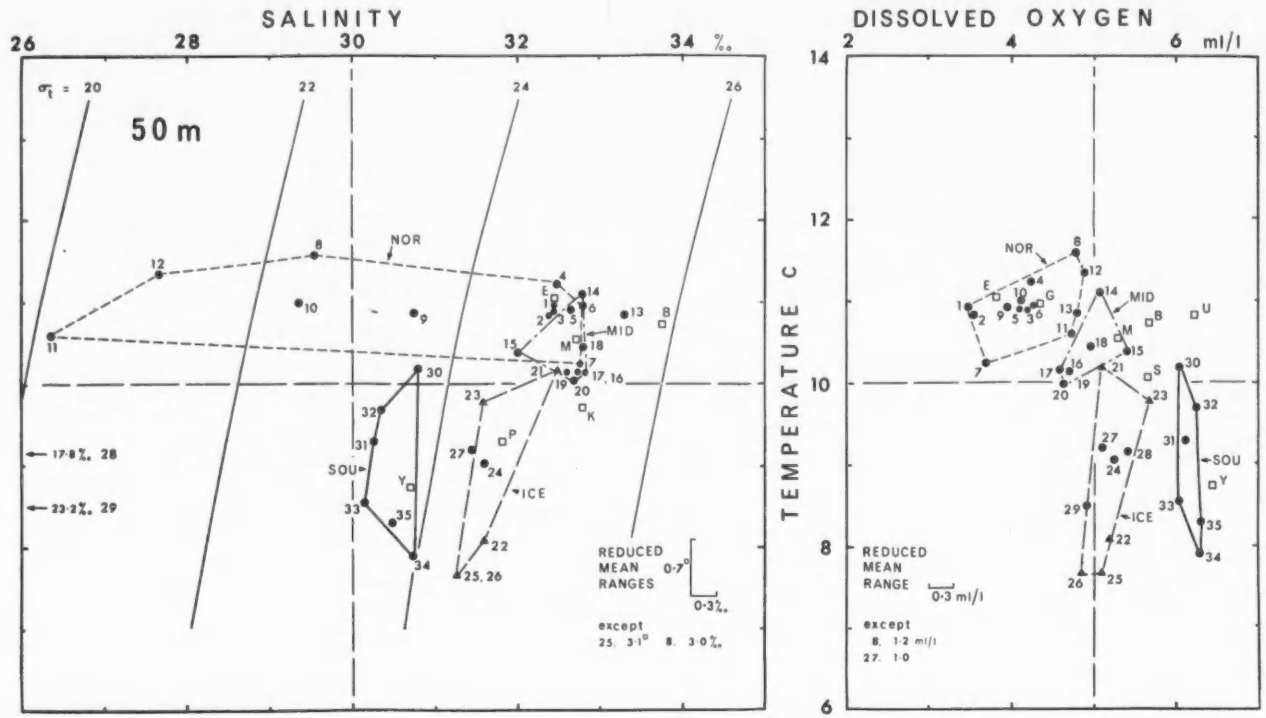

FIG. 9. Temperature–salinity and temperature–dissolved oxygen correlation plots for Chile inlets, Chile 70 cruise, 50 m depth.

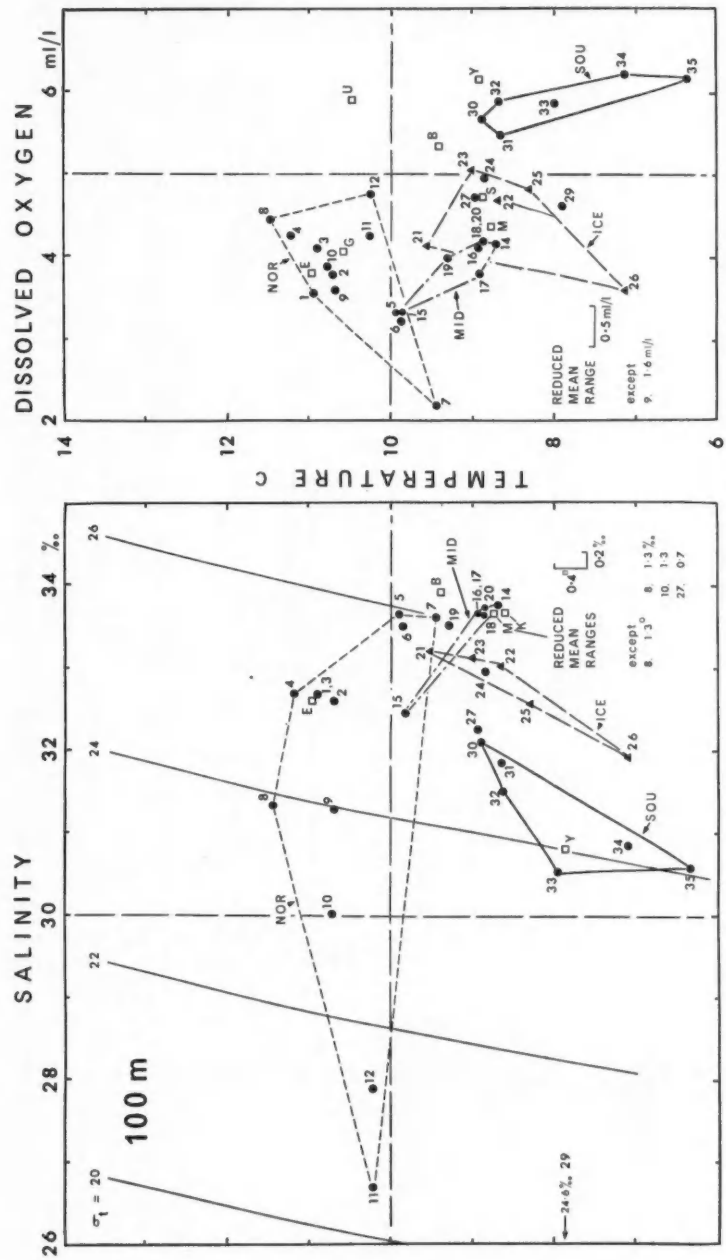

FIG. 10. Temperature–salinity and temperature–dissolved oxygen correlation plots for Chile inlets, Chile 70 cruise, 100 m depth.

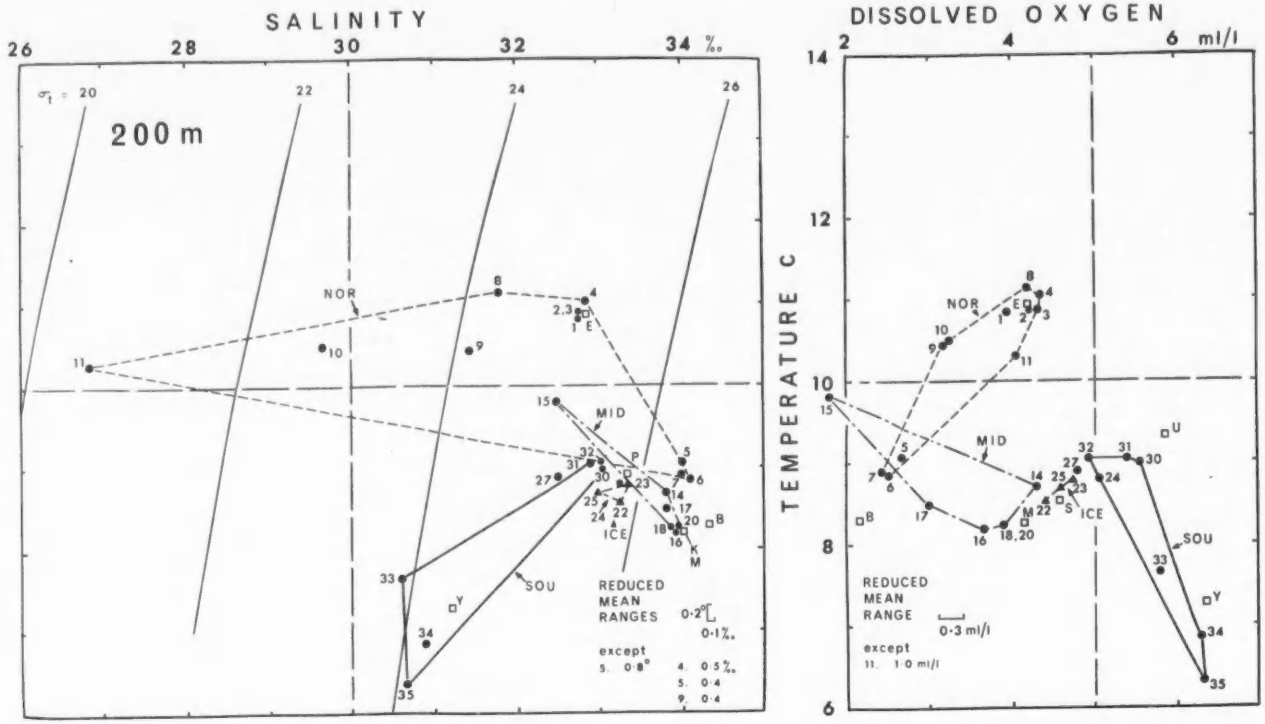

FIG. 11. Temperature–salinity and temperature–dissolved oxygen correlation plots for Chile inlets, Chile 70 cruise, 200 m depth.

TABLE 4. Surface and deep salinity values and salinity profile characteristics in inlets of Chile (Chile 70 cruise data).

		Salinity (‰)			Salinity–depth profile							
		Surface			Type			Depth of surface layer near inlet head (*m*)	Dimensions (*m*)			
									50%		90%	
	Inlet	Mo[a]	Hd[a]	Deep	Mo	Mi	Hd[a]		Mo	Hd	Mo	Hd
1	REL	26	4	32.8	2	2	1*b*	2	0	3	6	9
2	HOR	29	21	32.8	2	–	2	None	0	0	0	1
3	COM	30	28	32.9	2	2	2	None	0	0	0	<1
4	REÑ	31	28	32.9	2	–	2	None	0	0	0	<1
5	ELE 11–15	30	–	34.2	1*c*	–	–	–	0	–	10	–
6	JAC	29	24	34.2	1*c*	–	1*c*	7	0	6	23	14
7	PUY	22	16	34.0	2	2	1*b*	2.5	0	0.5	18	18
8	ELE 3–10	28	22	32.1	1*c*	1*c*	1*b*	7	0	0	8	7
9	AYS	24	4	31.4	2	2	2	None	0	1.5	13	12
10	QUI	27	20	29.7	1*c*	1*b*	2	2	0	0	0	4
11	FRA	18	7	26.9	1*b*	–	1*b*	1	0	2	10	12
12	PUE	–	20	27.9	–	1*c*	2	None	–	0	–	7
13	SQU	30	30	32.9	1*c*	–	2	None	0	0	0	0
14	JES	31	0	33.8	1*c*	–	1*b*	1	0	2	0	9
15	JUL	–	5	32.5	–	–	2	None	–	3	–	9
16	STE	1	4	33.9	1*b*	–	1*b*	3	4	4	20	20
17	MIT	1	<1	33.9	1*b*	–	1*b*	5	6	6	20	22
18,19	BAK	9	2	34.0	1*b*	1*b*	1*a*	3	2	4	11	25
20	NEF	3	–	33.9	1*b*	1*b*	–	–	3	–	23	–
21	ICE	3	–	33.3	2	2	–	–	3	–	22	–
22	EYR	10	6	33.4	2	1*b*	1*b*	8	2	6	19	22
23	EUR	10	<5	33.3	2	2	–	None	1	–	22	–
24	AND	21	<18	33.3	2	2	2	None	0	0	13	15
25	ASI	16	20	33.1	2	1*b*	2	None	0	0.5	22	25
26	PEE	–	9	31.9	–	–	1*b*	1	–	1	–	21
27	MON	23	<20	32.5	1*b*	1*b*	–	–	0	–	26	–
28	ULT	7	–	17.9	1*a*	1*a*	–	4	4	–	6	–
29	VAL	17	14	25.0	1*b*	–	1*b*	12	0	0	41	20
30	SWE	23	<7	33.2	1*b*	2	–	–	0	3	20	20
31	GAJ	24	20	33.1	1*b*	–	1*b*	10	0	0	28	28
32	XAU	28	22	33.2	1*c*	–	2	None	0	0	35	40
33	OTW	29	28	30.6	1*c*	1*c*	1*c*	45	0	0	0	10
34,35	ALM	30	28	30.8	1*c*	1*c*	1*c*	30	0	0	0	29

[a]Mo = mouth, Mi = middle, Hd = head of inlet.

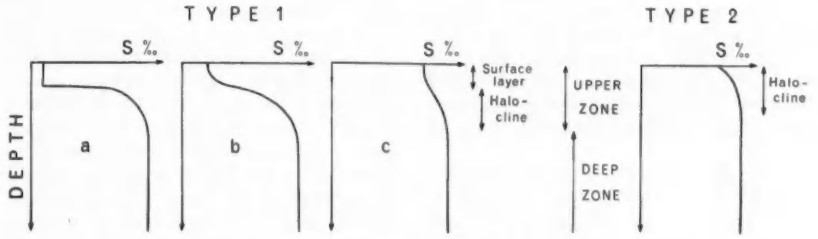

FIG. 12. Typical salinity–depth profile types in estuarine regions.

with strong wind conditions and was assumed to be real and due to evaporation at the surface. Later it was learned that the discharge for an evaporator, newly fitted for this cruise, occurred intermittently on the starboard side aft of where the water samples were taken and this must be assumed to be the cause for the high surface salinity values. The correlation with high wind conditions was probably real because the *Hudson* normally lies stern to wind on station and when the wind was strong enough and the surface layer density relatively high, some of the high salinity water discharged from the evaporator might not sink immediately and could contaminate the surface layer where the surface sample was taken.

Temperature–Depth Profiles

In contrast to the salinity–depth profiles, which generally showed an increase of salinity with depth, because salinity is the chief determinant of density in these waters, the temperature–depth profiles showed considerable variety in form. The basic one was of decrease of temperature with increase of depth but in many instances there were marked maxima and minima. The inlets having inflow of water from glaciers frequently had temperature–depth profiles with several maxima and minima.

Table 5 summarizes the information on temperature–depth profile types. These have been divided into two basic types: A, in which the temperature decreases monotonically with depth increase; and B, with one or more temperature maxima or minima. These have been divided into subtypes shown schematically in Fig. 13. For this purpose an extremum at the surface or bottom (e.g., in type A profiles) has not been counted as a maximum or minimum. The BT or STD records were used to determine the profile type; in the case of the B.3 type, this was the only way, as the maxima or minima were often so closely spaced that they would not be located when using a practical number of water bottles.

Table 5 has been arranged with the inlets in north to south order to show the tendency for types of profiles to group in geographical regions of the coast. The depth in meters of the upper isothermal layer in type A.2 profiles is shown in parentheses in the A.2 column of the table, and the maximum total number of maxima and minima in B.3 profile inlets is shown in parentheses in the B.3 column.

In identifying the temperature profile types on the BT or STD record, small scale maxima or minima (fine structure) of vertical dimensions less than 5 m were ignored. With this proviso it was found that in almost all instances the temperature–depth profiles in an inlet were of one type.

Temperature–depth profile types for the ocean and passage stations are shown by letters in Table 5 alongside the nearest inlet, and the information on possible glacier water contribution and ice occurrence has been repeated for convenience.

Dissolved Oxygen–Depth Profiles

The general trend in the dissolved oxygen–depth profiles was a decrease with depth from the surface. At the ocean stations there was a very pronounced subsurface minimum and a less pronounced deeper maximum; at the passage and inlet stations there were, in some instances, less pronounced maxima and minima in the profiles. The four basic types of oxygen–depth profile are shown schematically in Fig. 14, and Table 6 indicates the type of profile found at ocean, passage, and inlet stations.

At the ocean stations the oxygen–depth profiles (Type D) were characteristic of the south Pacific eastern boundary region and were significantly different from those for the passage and inlet stations. The major characteristic at the four ocean stations was a marked minimum of 2.0–3.9 ml/liter at 170–270 m depth, increasing to a maximum of 5.8–6.1 ml/liter at 550–600 m depth. A subsidiary minimum occurred at 30–50 m depth and a subsidiary maximum of 5.8–6.0 ml/liter at 80–100 m in some instances.

At the passage stations the surface values were generally slightly higher (5.5–7.5 ml/liter) than at the ocean stations (5.8–6.3 ml/liter) and below the surface the dissolved oxygen generally decreased, with most of the decrease in the upper 50 m. Most of the passage stations (type B) showed a slight oxygen minimum at 150–200 m where the value was 0.1–0.3 ml/liter less than that of the deeper water, and some of the passage stations (type C) in the middle region also showed a small oxygen maximum at 100 m or at 300–400 m. The deepwater oxygen values were generally 3.5–4.5 ml/liter in the northern and north middle region (stations E–P), 5.0–5.5 ml/liter in the south middle region (stations Q–T), and 5.5–6.5 ml/liter in the southern region (stations U–Y).

In the inlets the oxygen–depth profiles were similar to those at the passage stations, with surface values of 5.5–8.5 ml/liter and the main decrease extending over a greater depth to 50–200 m. Most of the inlets in the northern and southern regions had a minimum at 75–200 m (type B) where the values were 0.1–1.0 ml/liter lower than in the deeper water, whereas in the middle region the oxygen content generally decreased continuously to a uniform value in the deep water (type A). As in the passage stations, the deepwater values increased

TABLE 5. Types of temperature-depth profiles in Chile inlets, Chile 70 cruise. (Inlets by number, ocean and passage stations by letter.)

Temp profile type (see Fig. 13)					Glacial water inflow	Ice in water
A.1	A.2	B.1	B.2	B.3		
1 E	A(50)[a]					
2 F					V. small	
3						
4 G						
5	B(30)					
6						
	7(5)				Possible	
8						
9			9			
10	H(100)					
			11 I			
12	C(50)					
13						
J	D(55)	14			Yes	
				15(3)[b]	?	
				16(3)	Yes	
			17			
		18 K			Yes	Little
			19			
	L M(25)	20 N				
				21(3) O(3)	Yes	Yes
	Q(20)			22(7) P(4)	Yes	Much
				23(4) R(4)	Yes	Yes
				24(3)		
				25(4)	Yes	Yes
		S		26(3)	Yes	Yes
		27 T			Yes	
				28(3)	Possible	
		29				
		V	30	30(5) U(3)	Yes	
		W		31(4)	Yes	
				32(4)	Small	
X	33(3)					
Y	34(20)					
	35(20)				Yes	

[a]Depth in meters of upper mixed (isothermal) layer in type A.2 profiles.
[b]Number of maxima and minima, not counting top and bottom extrema, in type B.3 profiles.

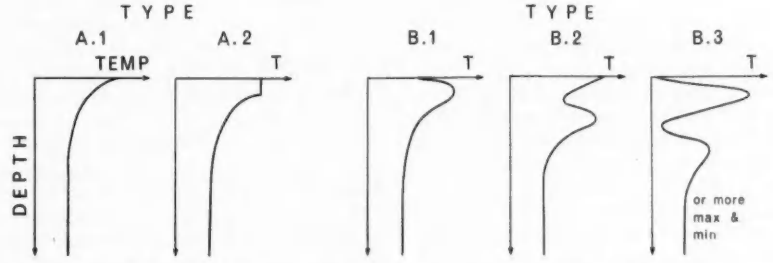

FIG. 13. Typical temperature–depth profile types in Chile inlets, Chile 70 cruise.

southward from 3 to 4 ml/liter in the northern and north middle regions, to 4.5–5.0 ml/liter in the south middle and 5.5–6.5 ml/liter in the southern region.

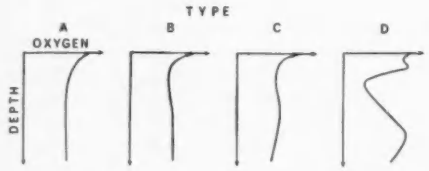

FIG. 14. Typical dissolved oxygen–depth profile types in Chile inlets, Chile 70 cruise.

TABLE 6. Types of dissolved oxygen–depth profiles in Chile inlets, Chile 70 cruise. (Inlets by number, ocean and passage stations by letter.)

Oxygen profile type (see Fig. 14)			
A	B	C	D
	1 E		A
	2 F		
		3	
G	4		
5		5	B
	6		
	7		
8	8		
	9	9	
10 H		10	
11	I	11	
		12	C
13			
14 J			D
15			
16			
17			
18 K		18	
19		19	
20 L	N	M	
21	O		
22	22	P,Q	
23		R	
24		24	
25		25	
26		S	
27	27 T		
28			
29			
	30 U		
31	W		
	32		
33	33	X	
	34 Y		
	35		

WATER PROPERTIES AT DEPTHS GREATER THAN 200 M

The depth of 200 m was selected as the maximum for the general display and comparison of water properties because there was information to this depth for more than 65% of the inlet stations (90% of the inlets). Values for 300 m or more were available for only 25% of the stations (only 40% of the inlets) and for 400 m or more for only 18% of the stations (only 23% of the inlets). In Table 7 are shown the changes in water properties beyond 200 m as the differences between the values at 400 and 200 m and between 600 and 200 m.

SECCHI DISC DEPTHS

Secchi disc depths were determined only at daylight stations, about 50% of the total, and as a result the distribution was rather irregular. However, they do show a broad trend, with the lowest average values in the north middle and iceberg inlet region and the highest in the north as follows:

	Inlets		Passage stations same regions	
	Mean & no. observations (*m*)	Range of values (*m*)	Mean & no. observations (*m*)	Range of values (*m*)
1 Reloncavi to 12 Puelma	6.3 (25)	0.5–11.5	7.8 (7)	3.5–12.5
14 Jesuitas to 26 Peel	2.1 (21)	0.1–7	1.9 (5)	0.8–8
27 Montanas to 35 Almirantazgo	5.0 (29)	0.5–9.5	7.1 (4)	6.5–9

The low values in the middle region (inlets 14–26) appeared to be due chiefly to very large amounts of glacial silt in the water.

Ocean stations B, C, and D were taken at night and no secchi depths were measured; at ocean station A off the north of the inlet region the secchi depth was 11 m. At seven ocean stations west and north of the inlets, in transit to Valparaiso after the inlet section of the cruise, the secchi depths were from 6 to 17 m, averaging 12 m.

Discussion

GEOGRAPHICAL DISTRIBUTION OF WATER PROPERTIES

The T-S correlation plots suggest groupings among the inlets. The data for the inlets in the northern region (inlets 1–12 in Fig. 7–11) form one group on the plots, whereas those for the

TABLE 7. Properties of deep water in inlets of Chile expressed as change in property from 200 to 400 m or from 200 o 600 m depth.

		Temp (*C*)		Salinity (‰)		Dissolved O^2 (*ml/liter*)		No. stations	
		200–400 m	200–600 m	200–400 m	200–600 m	200–400 m	200–600 m	200–400 m	200–600 m
				Ocean stations					
A	SPA4	−2.29	−3.21	+0.07	+0.05	+0.85	+1.73	1	1
B	GUA	−2.24	−3.29	−0.08	−0.11	+2.38	+3.48	1	1
C	ANN	−2.21	−3.07	−0.05	−0.07	+2.31	+3.26	1	1
D	TRE	−1.92	−2.55	+0.02	+0.01	+1.84	+1.94	1	1
				Passage stations					
L	MIL	−0.25	−0.40	+0.07	+0.11	0	−0.30	1	1
M	MID	−0.23	−0.40	+0.07	+0.11	−0.11	−0.40	1	1
O	ICY	+0.04		+0.12		+0.30		1	0
P	WID	−0.10	−0.23	+0.07	+0.09	−0.13	−0.25	1	1
Q	TRI	−0.14	−0.09	+0.10	+0.12	+0.10	+0.09	1	1
S	SAR	−0.23	−0.28	+0.10	+0.11	+0.24	+0.16	1	1
U	MAG	−0.98		+0.11		+0.12		1	0
V	SEA	−0.61	−0.66	+0.23	+0.23	+0.14	+0.19	1	1
W	LON	−0.43	−0.45	+0.23	+0.25	−0.08	0	1	1
Y	FAM	−0.16		+0.12		+0.08		1	0
				Inlets					
1	REL	0		+0.05		+0.25		2	0
3	COM	+0.10		+0.24		−0.56		3	0
5	ELE	0		+0.05		−0.17		1	0
6	JAC	−0.20		+0.03		+0.22		2	0
18	BAK	−0.23	−0.29	+0.07	+0.09	−0.04	−0.15	5	2
22	EYR	+0.05		+0.11		+0.18		1	0
24	AND	+0.03		+0.06		−0.39		2	0
25	ASI	0		+0.05		−0.09		1	0
30	SWE	−0.63		+0.17		+0.32		3	0
32	XAU	−0.41	−0.60	+0.23	+0.23	+0.31	+0.52	2	1
33	OTW	−0.25		+0.06		+0.20		1	0
35	ALM	+0.40		+0.12		−0.05		2	0

southern region (inlets 30–35) form a separate group at lower temperatures and with a much smaller salinity range below the surface (referred to again below). The north middle region inlets overlap the temperature range for the north and south regions at most depths, but have a much smaller salinity range below the surface, and the iceberg inlets form a separate group at most depths.

The temperature of the upper waters in the northern group of inlets is 3–5 degrees C higher than in the southern group and the average temperature difference in the surface water of the ocean outside the north and the south entrances to the passages and inlets is about 4.5 degrees C (Sverdrup et al. 1946, charts II and III, and USSR Ministerstvo Oborny 1953, charts 17 and 18). These differences correspond closely to the mean air temperature difference of 5 degrees C between the north and south (Fig. 6), suggesting that climatic conditions control the temperature in the upper waters. Below the surface layer the temperature difference between north and south decreases to only 2 degrees C at 20 m and less than 3 degrees C at 100 m and deeper. The properties of the subsurface waters (below about 20 m) in the inlets are probably determined more by those in the passages outside them (and these by the ocean water characteristics outside) than by the local climate. This suggestion is supported by the fact that the inlets that had glacier ice in them form a subgroup of the middle region with generally lower temperatures at the surface, and possibly at 5 m, but

the direct effect of the ice in reducing the surface temperature does not appear to extend much below 20 m depth.

The larger salinity range for the northern group of inlets than for the southern is because the former includes, toward the head of Elefantes (8), several inlets that are remote from the ocean source of water and are separated from it by relatively shallow sills.

On the T–O_2 plots, the northern and southern inlet group data points and the iceberg inlet (subgroup) data points form separate groups at all depths except 5 m (not shown here), whereas the remaining middle group inlets have properties intermediate between those for the northern and southern group. The separation on the T–O_2 plots is more marked than on the T–S plots: at all depths from 50 m and deeper, the northern, north middle, and southern inlet T–O_2 points fall into distinct groups with some overlap at 200 m due to Estero Julian (15), which has the lowest oxygen content of the inlets studied. The gradient of temperature from north to south contributes to this separation, as it does in the T–S plots, but the ranges of oxygen content emphasize the distinction between groups more than do the ranges of salinity.

Characteristic Diagrams

Although the T–S and T–O_2 correlation plots display the value of these water properties at set depths over the whole inlet region, they do not describe the water mass properties as a function of depth at specific locations. To investigate the relations between the water masses in the inlets, those in the passages, and those in the ocean, T–S and T–O_2 characteristic curves were drawn from the mean values for each inlet. These curves are too numerous to present independently here and become confusing when plotted together. Therefore, selected T–S and T–O_2 curves for the ocean and passage stations are shown in Fig. 15*a* and 16*a* and for the inlets in Fig. 15*b* and 16*b*.

T–S Diagrams

In Fig. 15*a*, six T–S curves have been chosen to represent the 4 ocean and 21 passage stations, and in Fig. 15*b* nine curves represent the 35 inlets. The group of stations or inlets represented by each curve is indicated on each figure. Curve B is taken to represent the ocean source water outside the passages and inlets. It is realized that the ocean water off the coast is likely to vary in properties with time as is evident from the differences between the T–S (and T–O_2) curves, even for the close-in stations B, C, and D, taken within a period of 1 week during this cruise. However, stations taken in 1967 from the U.S.N.S. *Eltanin* (Anon. 1969) in the vicinity of the present ocean station B (Guafo) show very similar T–S (and T–O_2) characteristics to those observed in 1970. It is particularly regretted that time did not permit taking ocean stations off the southern part of the coast (south of 47°S).

All the inlet water T–S curves are on the low salinity side of the ocean water curve and the differences in salinity between the inlet and ocean waters decrease with increasing depth, suggesting that the deep water of the inlets at the time of this survey had resided there long enough to suffer dilution by downward diffusion of fresh water from the river runoff and ice melt in the upper layers. The high salinity represented by curve 7 on Fig. 15*b* may indicate a recent inflow of ocean water to these three inlets, the mouth of Elefantes (5) and Jacaf (6) and Puyaguapi (7), which open in to it, as these three are close to the ocean.

There is no identity of deepwater properties in the passages with those in the ocean outside (Fig. 15*a*), and the limiting depth of exchange is therefore not immediately apparent. However, if one assumes that exchange between ocean and passages is predominantly along isopycnal surfaces, then the maximum density water in the passages (e.g., station M) must come from depths of less than 200 m in the ocean, probably 100–150 m, indicating the effective sill depths. This depth range is consistent with what can be determined from the charts.

A conspicuous feature of the passage station T–S curves is the considerable difference between the two for stations X and Y, the most easterly in the Estrecho de Magallanes, and all the remainder. (A corresponding difference in deepwater characteristics between inlets 33, 34, and 35, and the remaining inlets is evident.) Depths on the charts indicate a sill depth to the west of passage station X of more than 100 m but the differences in T–S (and T–O_2) curves between the neighbouring passage stations (W and X) on either side of the sill suggest a shallower depth. It is suggested that the source water for inlets 33, 34, and 35 (Otway and Almirantazgo) comes from the Atlantic through the eastern entrance to the Estrecho de Magallanes, whereas that for all the other inlets comes from the Pacific, or that the water in the eastern part of the Estrecho de Magallanes is sufficiently isolated by the sills at west and east that it can retain characteristics permanently modified by local conditions.

The progressive change in T–S curves along the northern region, indicated by those for inlets 2, 7, 9, and 11, emphasizes the effect of increasing distance from the ocean toward the head of Elefantes (8).

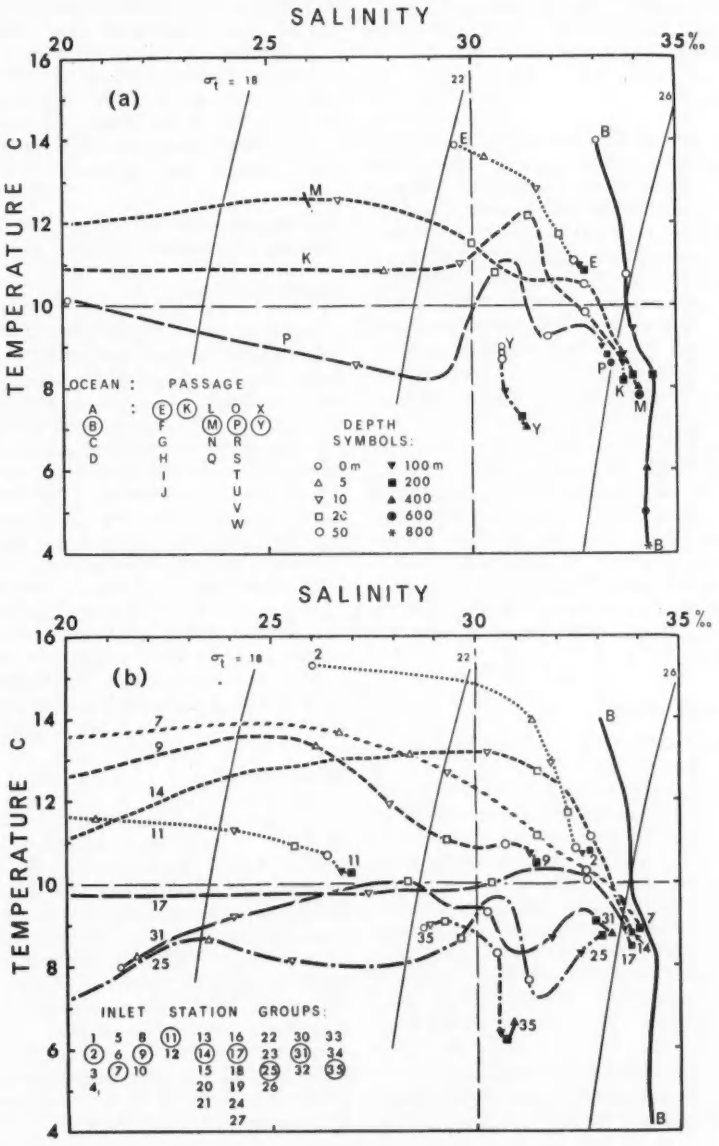

FIG. 15. Selected temperature–salinity diagrams to represent (*a*) ocean and passage stations, and (*b*) inlet stations, Chile 70 cruise. The group of stations or inlets best represented by each curve is shown on the figures with the type station circled.

Another dividing sill in the middle region is at the Angostura Inglesa between passage stations N and O (exemplified in Fig. 15*a* by curves for stations M and P).

T–O_2 Diagrams

It was more difficult to select typical T–O_2 curves than T–S curves, probably because some trend and possible separation is affected by the fact that the latter two properties determine density, which, as a rule, is arranged in an orderly fashion by gravity, but dissolved oxygen content does not significantly affect density. Figure 16*a* shows the T–O_2 curves selected to characterize the ocean and passage waters. For the inlets, the best that could be done was to draw envelopes of T–O_2 curves for groups of inlets (Fig. 16*b*). These groups were suggested by geographical position, and by the relation of inlets to passage stations.

A sequence of inlet and passage station characteristics, similar to the geographical sequence demonstrated by the T–S and T–O_2 correlation plots for constant depths, is again evident. The T–O_2 characteristic curves in the passages (except X, Y) show some similarity to ocean water characteristics above the oxygen minimum, which is a feature of the Pacific waters off South America and is at about 200 m depth close to the coast of southern Chile.

Deeper Water Properties

At the ocean stations the water temperature continued to decrease below 200 m (Table 7) because the main thermocline continued to below 800 m here, but the salinity changes were small and variable in sign because the salinity reached a weak maximum at about 250 m (from bottle casts and STD records). The oxygen content increased below 200 m because the oxygen minimum occurred at about 150–200 m. In the passages, the salinity increased with depth, the differences being larger than in the ocean, which is consistent with the passage water coming from well above the salinity maximum outside, i.e., from depths of less than about 200 m.

In the inlets the salinity increased with depth beyond 200 m. The temperature generally decreased with depth with the larger decreases being in the southern group (e.g., 30 and 32) at the west end of the Estrecho de Magallanes. These observations in the passages and inlets are consistent with the observed property distributions in the ocean, but the relatively large increase in oxygen content with depth in the southern inlets appears inconsistent with the water coming from depths of less than 200 m outside where the oxygen generally decreased to this depth. However, at some ocean stations (e.g., Guafo, B) there was a subsidiary oxygen minimum at 30 m and a maximum at 75–100 m. This gave rise to an increase in oxygen content with depth in the upper layer, which is probably the source of the inlet waters and could explain the increase with depth found inside if it is typical of the ocean water off the southern coast.

Comparison between the Inlets of Chile and those of British Columbia and Alaska

Geographic features

If the northeast Pacific inlets (British Columbia and Alaska) are combined as a group, then their mean length and width are almost the same as those studied in Chile, but, as seen in Table 1, the Chile inlets fall between the British Columbia and Alaska ones as separate groups. In depths the three regions are similar in mean values.

In Table 1, 14 inlets each in Chile and in the northeast Pacific are listed as having glaciers that definitely or probably contribute melt water. This number is believed to be the total in the northeast Pacific but maps and charts indicate that there are probably twice as many in Chile as the 14 studied during this cruise. Also, there are undoubtedly others than the six studied in Chile that have glacier ice in their waters. The indirect and direct influence of glaciers on the oceanography of the inlets is greater in Chile than in the northeast Pacific.

Water properties

The data obtained on water characteristics during the Chile 70 cruise are compared, by means of T–S and T–O_2 envelope diagrams, with data obtained by the University of British Columbia in the inlets of British Columbia and Alaska. In a previous paper, Pickard (1967, fig. 17) compared the waters of the latter two regions by means of such diagrams, which showed the envelopes of the points representing the combinations of mean values of these properties along inlets. It was shown that the waters of the southern British Columbia inlets, which are the lowest latitude group in the fjord coast of the northeast Pacific, were generally warmer and less saline than those of northern British Columbia, whereas the properties in the Alaska inlets overlapped the latter group but extended to slightly lower temperatures and higher salinities. The inlets with glacier ice in the water (occurring only in Alaska) formed a separate class with intermediate salinities but distinguished from the others by generally lower temperatures.

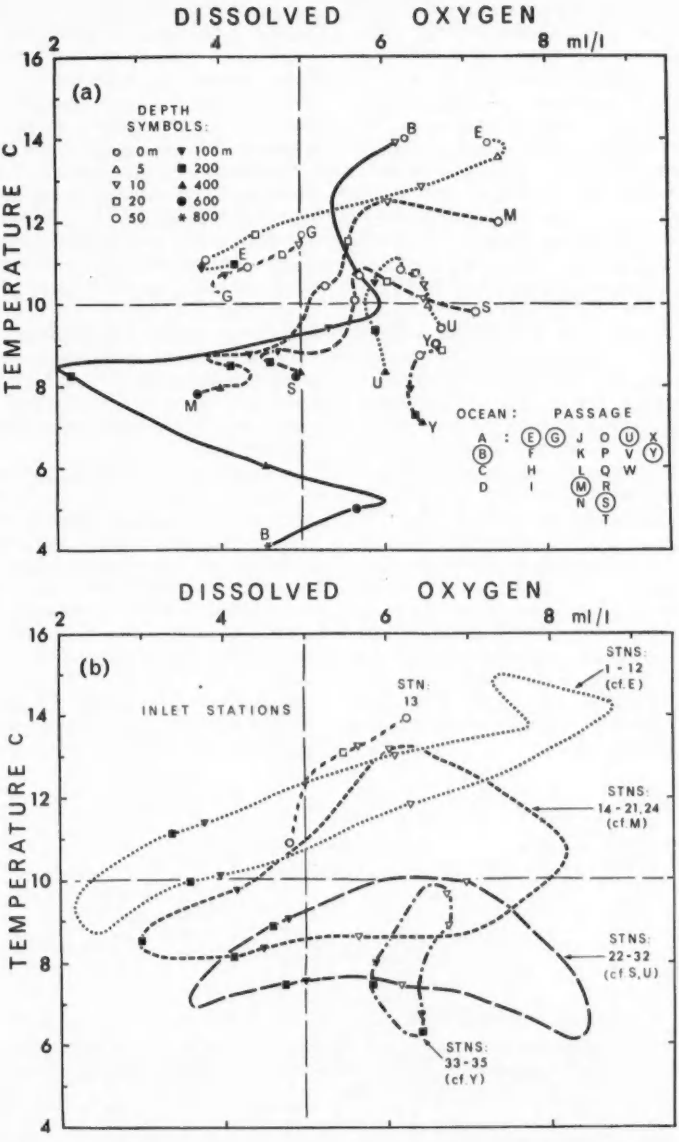

FIG. 16. (*a*) Selected temperature–dissolved oxygen curves to represent ocean and passage stations, and (*b*) envelopes of temperature–dissolved oxygen curves for groups of inlets with an indication of average depth at 10, 100, and 200 m, Chile 70 cruise.

For comparison with the Chile inlets, T–S and T–O_2 envelope diagrams are shown for the surface, 20-, 50-, 100-, and 200-m depths in Fig. 17–19. Note that in Fig. 17 the ranges of temperature, salinity, and oxygen are different from those in the other two figures. The northern British Columbia group, which is similar to the Alaska group, has been omitted to simplify these diagrams and the British Columbia data have been brought up to date to 1969. When the Chile and the British Columbia/Alaska regions are being compared, it should be remembered that, as these are in opposite hemispheres, the northern Chile and southern British Columbia inlets are the lower latitude groups, whereas the southern Chile and Alaska groups are the higher latitude ones (Fig. 1). In addition, the latitude ranges for the southern and northern hemisphere groups are not the same, the Chile inlets being generally about 6° lower in latitude than the British Columbia/Alaska ones (see Fig. 18). The southern British Columbia inlets, group A, are smaller ones in the extreme south and have shallow sills, which account for the lower salinities and higher temperatures in this group.

When the envelopes are being compared, it should also be noted that although the Chile data are from a single cruise in the early fall, the British Columbia/Alaska data are from as many as 10 cruises per inlet from 1951 to 1969, although only summer data have been used for the present diagrams. Therefore, if some of the British Columbia/Alaska data envelopes are larger than Chile ones, this may be attributable to year-to-year variations introducing additional scatter.

In Fig. 17, for the surface (or near-surface values for oxygen in some British Columbia inlets where surface values were not available) the temperature scale covers 3–20 C, salinity from 0 to 35‰, and oxygen from 5 to 11 ml/liter. In temperature, the most obvious feature is that the southern British Columbia group covers a larger range than the northern Chile group, although the latter extends over a larger latitude range (5.5° of latitude compared with 3°), but this may simply be a result of there being more data for British Columbia. The mid-Chile group with a latitude range of only 1.5° covers a larger temperature range than does the more extensive northern Chile group, but has a lower mean temperature for a lower latitude than the southern British Columbia group. The southern Chile group has a small temperature range and a lower mean value than for the Alaska inlets, although the former are at 4° lower latitude on the average. The Chile iceberg inlets are colder at the surface than the Alaska ones.

In salinity at the surface, the lower latitude inlets in both hemispheres have a full range of values from near zero to 30‰, whereas the higher latitude group values are in the upper half of this range, except for the Chile iceberg inlets, which are much less saline than the Alaska ones. The latter is taken as a significant difference between the two regions, as the Alaska data were collected at a comparable time of year during or just after the maximum river runoff.

In oxygen values at the surface, the Chile and British Columbia/Alaska inlets have about the same mean values, but the Chile iceberg inlets have

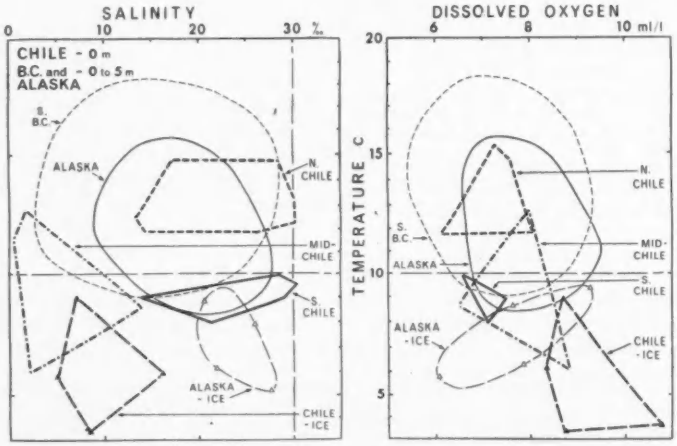

FIG. 17. Envelopes of temperature–salinity and temperature–dissolved oxygen characteristics for Chile and for British Columbia/Alaska inlets, surface or near surface values. ("ICE" denotes inlets with icebergs.)

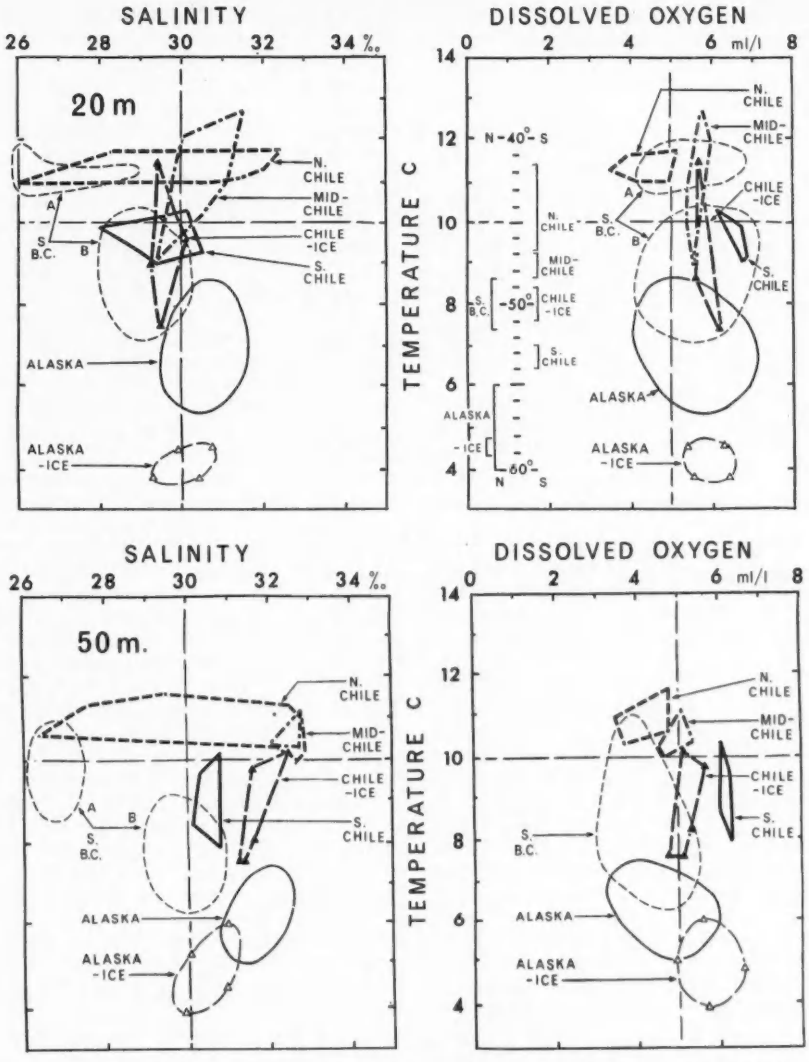

FIG. 18. Envelopes of temperature–salinity and temperature–dissolved oxygen characteristics for Chile and for British Columbia/Alaska inlets, 20 and 50 m depth. ("ICE" denotes inlets with icebergs.)

higher values than the Alaska ones. (The suggestion has been made that the higher silt content of the water in these inlets than in Alaska might influence the measurement by the Winkler method, but no specific reasons are suggested.)

For the subsurface levels, I will first compare the water properties individually, referring to Fig. 18 and 19, in which the temperature range is reduced to 3–14 C, salinity to 26–35‰, and oxygen changed to 0–8 ml/liter. The temperatures of the northern and mid-Chile inlets are highest, the temperatures in the southern British Columbia inlets (at 48–52°N) are similar to those in the southern Chile ones (at 52–54°S), and the temperatures in the Alaska

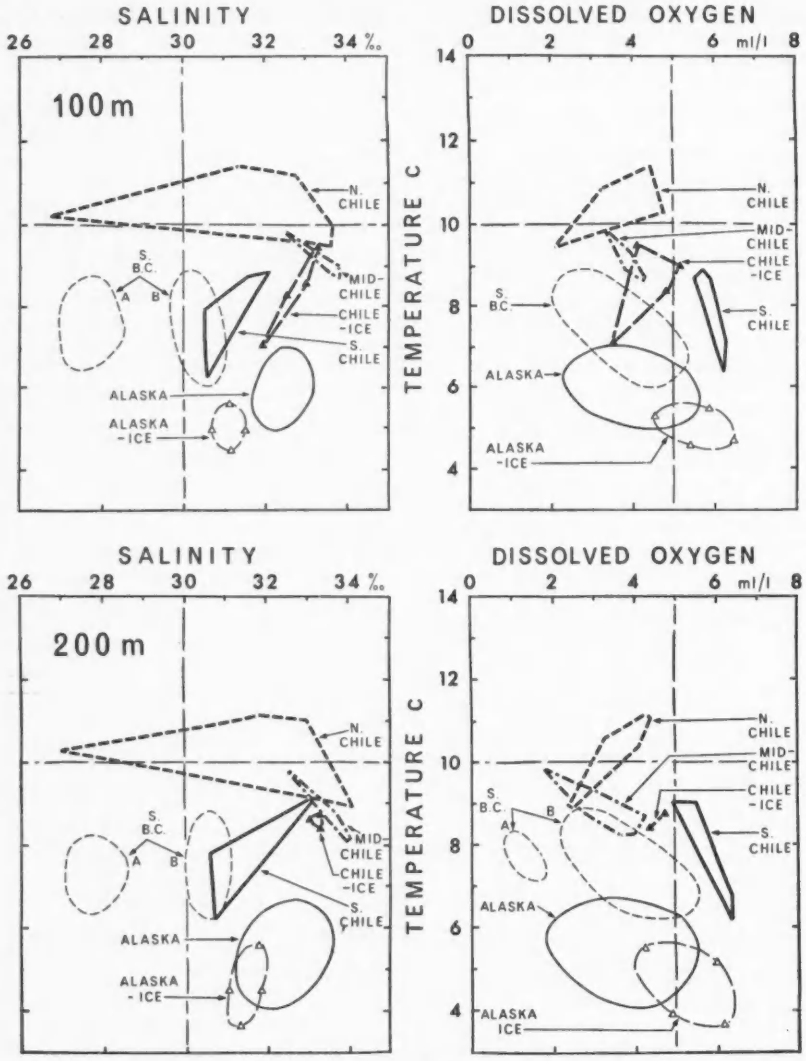

FIG. 19. Envelopes of temperature–salinity and temperature–dissolved oxygen characteristics for Chile and for British Columbia/Alaska inlets, 100 and 200 m depth. ("ICE" denotes inlets with icebergs.)

inlets are lower than these by 2–3 degrees C. In contrast to the surface situation the Chile iceberg inlets at 48–51°S are warmer by 4–5 degrees C than the Alaska ones at 57–59°N. Below 20 m depth the ranges of temperature for the groups compared above are substantially the same, except that above 200 m the Chile iceberg inlets show a larger range of temperatures than the Alaska ones.

The salinities below the surface for the northern Chile group (one-third of the total) extend over a range of 6.5‰, encompassing the values for all the other Chile inlets and almost all the northeast Pacific ones. This large salinity range is chiefly due to the low values in the inlets 10, 11, and 12

toward the head of the long Elefantes system (inlets 5 and 8). In contrast, the mid-Chile group, which has more direct and deeper connection with the Pacific, has a salinity range of 2.5‰, or less.

The oxygen content below the surface has the same overall range of values in Chile as in British Columbia/Alaska to 100 m depth; below this, a few of the British Columbia inlets have lower values (to less than 1 ml/liter) than observed anywhere in Chile. A tendency for oxygen values to increase with latitude in Chile is the other feature of this region.

Now I use the characteristic diagrams for their main purpose, to display the combinations of properties that occur. At the surface, the T–S combinations are so diverse that the envelopes overlap for most of the regions, both north and south of the equator. The exceptions are the iceberg inlet envelopes, both for T–S and T–O_2, which are distinct from the other groups and distinct between Chile and Alaska, and the southern Chile envelopes, which are at the lower end of the temperature range of the Chile noniceberg inlets.

At 20 m there is some overlap between the T–S envelopes for the four Chile inlet groups, but deeper than this they are substantially distinct. For T–O_2 the envelopes for the contiguous mid-Chile and iceberg groups overlap at 20 m but are distinct at most greater depths. The T–S envelopes for the Chile inlets are separate from those for British Columbia/Alaska because both the temperature and salinity values are generally higher in Chile, and the T–O_2 envelopes are separated chiefly because of the higher temperatures in Chile. The temperature and salinity differences between the Chile inlets and the British Columbia/Alaska ones below the surface are in the same directions as the values in the ocean outside to about 200 m (Fig. 20). There is no apparent correlation in dissolved oxygen content for which the range of values in the two inlet regions is much the same, although the ocean water outside Chile has lower oxygen values in the upper 200 m than that outside British Columbia/Alaska.

Secchi disc depths in the inlets in Chile and in the northeast Pacific have the same range of values from a fraction of a meter near sources of glacier runoff to 10 m or more in the outer passages. A notable exception was that in Baker (18) secchi depths of only 1 m (due to glacial silt) were observed up to 80 km from the melt-water inflow, whereas in the northeast Pacific such low values are not found more than 15–20 km from the inflow. The Baker system was also a region of much lower plankton concentration than elsewhere and of few birds (field observations during Chile 70 by B. McK. Bary and R. B. Brown respectively).

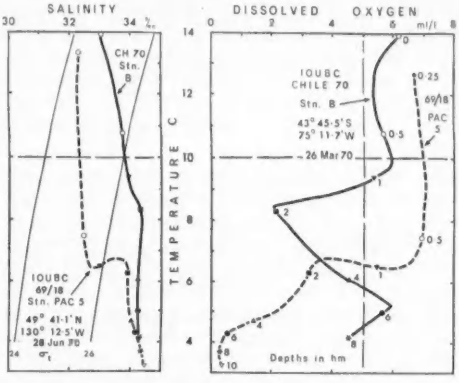

FIG. 20. Temperature–salinity and temperature–dissolved oxygen curves for typical ocean stations off Chile and off British Columbia.

Conclusions

The Chile 70 cruise included 32 inlets of 15–300 km length, averaging 66 km, in the mainland and Tierra del Fuego coasts of southern Chile. From the charts it is estimated that there are at least another 35 inlets of 15 km or more length in these coasts and 50 or so of 15 km or more length in the islands west of the mainland coast. This count does not include passages that open at both ends to the sea. Of the unstudied inlets, the largest (Skyring) approaches 100 km length, 10 are of 40–50 km length, and the rest are shorter. Although the studied inlets comprise only about one-half of the total in the mainland coast, they do include the majority of the longer ones and I venture to suggest that they provide a representative picture of the inlet characteristics of the mainland coast of Chile.

As determined during the Chile 70 cruise, this picture is of a typical fjord or deep estuary coast, with a basic two-layer water system of a shallow low-salinity layer over more saline deep water, the upper-layer low salinity being due largely to river runoff. The direct effects of the glaciers in contributing rock flour and icebergs to the water are more extensive in Chile than in British Columbia/Alaska, and complicated temperature structures in glacier and iceberg inlets are a feature of the Chile coast. From charts and maps, it is estimated that as many as 15 more Chile inlets than the 15 studied may have significant glacier water runoff and possibly 5 of these may have glacier ice in addition to the 6 studied.

Stagnant basins are uncommon and this may well be another feature of the Chile inlets. The absence of shallow sills is the main reason for this

in the inlets studied. A potentially interesting region, which was not practical to study during Chile 70, is the deep basin in the west end of Skyring Water (northwest of Otway, inlet 33) where there are depths of 600 m or more. The sill depth from Otway is about 10 m but there is a narrow and shallow connection at the west to Gajardo (31) with reported strong currents that might bring in saline water to refresh the deeper layers.

In the islands off the mainland coast the majority of the inlets appear to have direct connection to the ocean waters outside, and this, together with the small watersheds and small stored runoff areas, suggests that these bodies would generally have higher salinity water, close to oceanic values, and lack the low salinity surface layer characteristic of the estuarine structure of the inside inlets.

Suggestions for future studies of the physical oceanography of the inlets in Chile include: (1) observing at different times of the year in selected typical inlets to determine the variations in water properties through the year; (2) devoting particular attention in this respect to the glacier inlets to determine the reasons for their complex temperature structure; (3) comparing the seasonal variations in the inlets with glacier water runoff only, such as Steffen (16), with those having icebergs as well, although the latter will present serious difficulties unless a vessel strengthened against ice is available; (4) extending observations to the unexplored mainland inlets as opportunity offers; (5) gathering information on river runoff and precipitation, particularly in the middle coast region, to relate to the freshwater distribution in the inlets as indicated by the salinity decrease below that of the ocean source water; (6) examining a selection of the inlets in the islands to see if they have any unanticipated characteristics.

Acknowledgments

I express my thanks to many organizations and individuals who made this study possible: to the Director, Atlantic Oceanographic Laboratory, Marine Sciences Branch, Canada Department of Energy, Mines and Resources, for making time available to the University of British Columbia for this investigation in Chile during the *Hudson* 70 Expedition; to the Chilean Government for permitting us to make these detailed oceanographic investigations in their inshore territorial waters and for their assistance in making arrangements for the cruise through Capitan R. Herrara, Director, Instituto Hidrografico, Armada de Chile and Sr H. Sievers, Head, Centro Nacional de Datos Oceanograficos de Chile; to the Chilean scientists who took part in the cruise and assisted in the physical oceanographic observations (Prof H. Inostroza, Drs L. Chuecas and F. Rey, Srs R. Montaner, B. Uccelletti, and N. Silva); to Drs W. V. Burt and G. S. Pond of Oregon State University for their assistance during the cruise; to Mr I. W. Duedall of the Marine Ecology Laboratory, Fisheries Research Board of Canada, for making available facilities for determining dissolved oxygen and nutrients and taking part in the field work; to Mr P. Wadhams (A.O.L.) for computing on board; to Mr J. Pilote (A.O.L.) for all the hydrographic sounding; to Dr B. McK. Bary (U.B.C.) as Assistant Chief Scientist; and particularly to Mr M. P. Storm of this Institute for preparing the field equipment, being responsible for the salinity determinations, and for assembling the data report. Especially, I thank Captain D. W. Butler, Master, C.S.S. *Hudson*, and his officers and crew for their generous assistance in all phases of the field work and for their skill in handling the ship and boats, particularly in confined and incompletely surveyed waters. The support of the National Research Council through Grant A.5091 is gratefully acknowledged.

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A Computer Simulation of the Effects of Superimposed Mortality Due to Pollutants on Populations of Fathead Minnows (*Pimephales promelas*)

WILLIAM T. WALLER,[1] M. L. DAHLBERG,[2] RICHARD E. SPARKS,[1] AND JOHN CAIRNS, JR.[1]

WALLER, W. T., M. L. DAHLBERG, R. E. SPARKS, AND J. CAIRNS, JR. 1971. A computer simulation of the effects of superimposed mortality due to pollutants on populations of fathead minnows (*Pimephales promelas*). J. Fish. Res. Bd. Canada 28: 1107–1112.

This computer simulation study tested the effects of 99 levels of proportional mortality on fathead minnow (*Pimephales promelas*) populations, assuming that the parent–progeny relationship is of the form proposed by Ricker. Limited data from two studies on three lakes were available to fit the model and obtain estimates of parameters for fathead minnow populations. Based on these estimates, 25 replications of a 50-generation cycle were simulated under two levels of environmental variability. Arbitrary extinction levels of 5, 100, and 500 females had little effect on the results. Increasing environmental variation lowered the percentage mortality at which population extinctions occurred. In general, the results are compatible with the recommendation of the U.S. Committee on Water Quality Criteria, that the maximum concentration of zinc to which fish could be continuously exposed should not exceed $\frac{1}{100}$ the 96-hr TL_m (median tolerance limit) — a concentration that caused a 50% reduction in the mean number of eggs laid per female by fathead minnows in a laboratory study.

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A computer program was designed to simulate the effects of various reductions in egg production on the population size of fathead minnows through time. Although the simulations show the effect of any superimposed mortality, the study was prompted by a recommendation in the report of the Committee on Water Quality Criteria (Anon. 1968) that the maximum concentration of zinc to which fish could be continuously exposed should not exceed $\frac{1}{100}$ the 96-hr TL_m (median tolerance limit). This recommendation was based on the then unpublished work of Brungs (1969), who attempted to determine the maximum acceptable concentration of zinc for the fathead minnow (*Pimephales promelas* Rafinesque). He felt that the concentration determined safe by his study might only be applicable to waters equivalent in quality to the experimental water used. Brungs asserted that the test fish were not subjected to the usual stresses associated with natural environments, and that further studies were being carried out to determine the influence of both environmental stresses and supposedly safe concentrations. Brungs concluded that if a 20% reduction in egg production is biologically insignificant in terms of sustaining the population, then the application factor would be $\frac{1}{200}$ the 96-hr TL_m value, and if a 50% reduction is insignificant, then the application factor would be $\frac{1}{100}$ the 96-hr TL_m value.

Life History Information

Life-history studies have shown that the number of age II individuals in natural populations of fathead minnows is relatively small. Isaak (MS 1961) found that most age I fathead minnows in a shallow lake died upon completion of spawning, and Markus (1934) found only 15–20% of the population reached age II. Markus (1934) speculated that fish that survived to age II did not take active part in either of the previous spawning periods and that enabled them to survive through the winter.

Information from two studies (Isaak MS 1961; Andrews MS 1970) on three lakes that were not managed for minnow production was available for estimating the relation between the abundance of spawning females and the resulting abundance of female progeny. The data from the studies of Isaak and Andrews are as follows:

[1]Biology Department and Center for Environmental Studies, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA.

[2]U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Narragansett, Rhode Island 02882.

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	Spawners	Returns
Isaak MS 1961	60831	9010
	9010	26308 (estimated)
Andrews MS 1970	5596 (warm water)	25000
	27867 (cold water)	20000

Isaak did not determine the number of female progeny from the spawners in the 2nd year; however, we estimated the number to be 26,308, based on mortality in the previous year. Two assumptions regarding the life history of fathead minnows were made in the simulation: the sex ratio is typically 1:1, observed by Carlson (1967) and Isaak, and female minnows live only to age I and die after spawning.

The Model

The life-history pattern of the fathead minnow was the primary consideration in selecting a mathematical model to simulate population abundance in successive generations. This relatively simple pattern — egg to fry to adult, and death following spawning, in biennial cycles — is analogous to that in Pacific salmon. A model proposed by Ricker (1954) and later changed in form (Ricker 1958) was selected on the basis of its utility in describing the spawner–return relations of Pacific salmon stocks (Dahlberg MS 1968). Paulik and Greenough (1966) gave the derivation of Ricker's compensatory spawner–recruit model (the notation is modified from Ricker's original formulation). Briefly, the assumptions of a freshwater habitat of fixed size and strict compensatory mortality (exponentially decreasing) lead directly to the model:

$$R = \left\{\alpha S e^{-\beta S}\right\} e^{\epsilon},$$

where R = total return from S number of spawners; S = number of spawners in parent year; α = parameter estimated from empirical observations; β = parameter estimated from empirical observations; ϵ = error term associated with the model, log-normally distributed, with mean zero and variance σ^2.

The importance of the multiplicative error term is seldom mentioned by fishery scientists fitting the model. Ricker (1958) stressed that losses due to the physical environment tend to act in a multiplicative fashion, i.e., a fraction rather than a fixed number of the population is lost. When an additive error term is assumed, the parameters of the model must be estimated by nonlinear regression techniques. However, the multiplicative model is easily fitted by dividing each side of the equation by S and then taking natural logarithms. Then the model becomes linear:

$$y = \ln(R/S) = \ln\alpha - \beta S + \epsilon$$

and the method of least squares may be used to estimate the parameters.

Results and Discussion

Figure 1 shows the recruitment curve from the Ricker model fitted to the values given above and the 90% confidence interval for each of the plotted points. The test of significance, proposed by Ricker (1958), and confirmed by Tanaka (1962), showed that the fit of the curve to the values used for the simulations was relatively good — $F = 3.6$ with 3 and 2 df; the coefficient of determination was 0.98. A replacement line drawn through the origin at 45° to the abscissa intersects the curve at the equilibrium level, or replacement stock density. At this point on the curve, the spawning population would, theoretically, just maintain itself from generation to generation if environmental effects could be removed. The number at which this constancy would occur (replacement density) for the estimates we used was 27,182 females.

To determine what effect the estimated number of returns from 9010 spawners had on the values of α and β, two estimates of these parameters were obtained. The first estimates were based on all values used and the second estimates were based on observed numbers of fish; the value for returns estimated from Isaak's (MS 1961) study and its associated spawner value were excluded in the

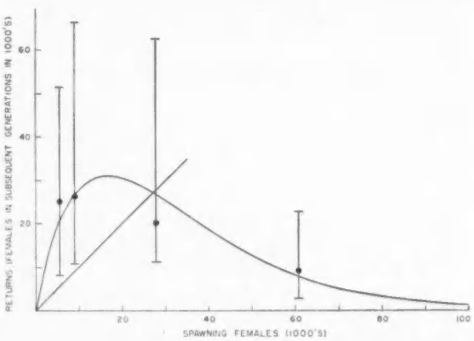

FIG. 1. Recruitment curve from Ricker model fitted to data from Isaak and Andrews (MS 1970).

second estimates. The results showed that the difference between the two sets of α and β values determined in this manner was less than 2%, and because of this small difference the estimates based on all values were used for all simulations. The estimates used in the simulations and their upper and lower confidence limits are given in Table 1.

The results of the simulations are dependent on the estimates of α and β. To determine the influence of these parameters on the number of extinctions, sets of all possible combinations of the upper and lower confidence limits for α and β were used in the simulations. Examination of Table 2 shows that the number of extinctions was not markedly influenced by the different β values. The marked influence of α on the number of extinctions was to be expected. Since α determines the maximum equilibrium rate of exploitation in the deterministic case (no random flunctuation), it is not surprising that α still determines the maximum rate of exploitation in the stochastic case.

Figure 2 shows the time path of the number of females for two simulated populations of fathead minnows in which no environmental variation or superimposed mortalities were added. The broken line represents an initial population with fewer than the equilibrium number of females. The solid line represents an initial population with twice the equilibrium number of females. Both populations stabilized near the theoretical equilibrium (dotted line) in approximately 10–12 generations. Based on these results, all additional

TABLE 1. Estimates of population parameters from Ricker model.

Parameters	Estimates	95% confidence interval extends from	to
α	5.15	2.15	12.30
β	6.03×10^{-6}	3.46×10^{-6}	8.60×10^{-6}
ϵ	0.262		

TABLE 2. Number of extinctions for two levels of environmental variation as influenced by α and β.

	1×SD		2×SD	
	α upper	α lower	α upper	α lower
β upper	201	1195	224	1328
β lower	216	1286	257	1524

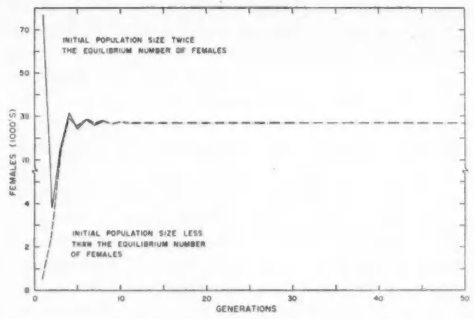

FIG. 2. Time path of the number of females for two simulated populations of fathead minnows, in which no environmental variation or superimposed mortalities were added.

simulations started with an initial population size equal to the equilibrium number of females. This starting point was chosen because, in a natural population in which superimposed mortalities might occur, the number of females would probably fluctuate about the replacement stock density.

An actual population might be smaller than predicted because of environmental fluctuations, which in this study were represented by the error term (ϵ). By assigning the error term extreme values, we were able to assess the effect of environmental extremes. Figure 3 shows the time path of the number of females for two populations of fathead minnows, each with a starting population size of 27,182 females. In each of these simulations the effect of environmental variation was added at

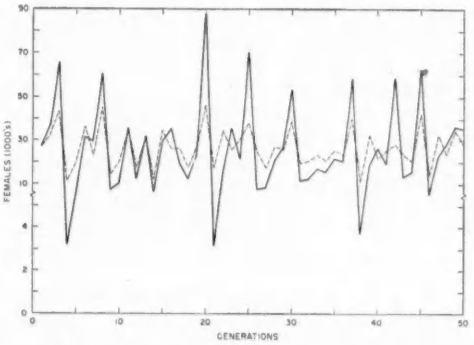

FIG. 3. Time path of the number of females for two simulated populations of fathead minnows, in which environmental variation was equal to ±1 SD of the mean error term in one simulation, and ±2 SD of the mean error term in the second simulation.

each generation. Mathematically, environmental variation was represented by a log-normal random variate and a multiplier that was applied to the standard deviation of the error term. A computer subroutine generated random numbers that were log-normally distributed, with a mean equal to zero and a standard deviation equal to the estimated standard deviation of the error term (Martin 1968). A positive random number represented favorable environmental conditions that caused the population size to increase, whereas the reverse was true of a negative value. By using random numbers that were log-normally distributed, large positive or negative values would rarely occur. This would simulate the rare occurrence of environmental extremes. In a second run on the computer, the subroutine generated values that had a standard deviation twice that estimated from the original data. The second run simulated increased environmental variability, and the effect can be observed in the increased oscillation about the equilibrium level in Fig. 3. The simulated population in which the environment varied within ±2 SD of the mean error term had a mean population size of 26,964 and the population that varied within 1 SD of the mean error term had a mean population size of 27,047. Both values are reasonably close to replacement stock density (27,182) in the deterministic case.

Superimposed mortality was simulated by applying a fixed percentage mortality to the population of females each generation, and subtracting the calculated number before the next spawning cycle. This was assumed to have the same effect as reducing the egg production by a fixed percentage each generation. Mortality rates were increased from 1 to 99% in increments of 1%. At each level of mortality the initial population size was the equilibrium number 27,182. The population was reduced by the percentage mortality, and the Ricker model, including the random error term, was used to predict the number of females in the second generation. The number of females remaining in the second generation was then used to predict the number in the third generation, and so on, for 50 generations. If the population size in any generation before the 51st consisted of fewer than 500 females, the computer tallied an extinction and the prediction cycle terminated.

The choice of 500 females as the level at which extinction occurred was based on the following considerations: most organisms require a certain population density in order for mates to find each other, for social potentiation to occur, to maintain sufficient genetic variability in the population, etc. Five hundred would probably be a minimal population for minnows in a lake; certainly, 500 fathead minnows is below the lower detection limits of the sampling methods employed by Isaak (MS 1961) and Andrews (MS 1970). However, to see how arbitrary extinction levels affected the results, all simulations were run at extinction levels of 5, 100, and 500 females, and the total number of extinctions at each level is shown:

	$1\times\epsilon$	$2\times\epsilon$
5 females	426	437
100 "	468	502
500 "	513	594

Because of the small increases in the total number of extinctions that occurred as the extinction level was increased from 5 to 500 females, the results presented are based on an extinction level of 500 females.

Environmental conditions (simulated by the sequence of random errors) were the same for all levels of mortality. The computer then generated another set of environmental conditions, and the simulations at all levels of mortality were repeated. The same set of 50 random numbers for each of 99 levels of mortality was used to enable us to segregate the effect of mortality level from the effect of the random sequence. Twenty-five sets of environmental conditions were simulated with the error term varying between ±1 SD. The degree of environmental variation was then increased by allowing the mean error term to vary between ±2 SD for 25 additional sets of environmental conditions. In summary, we simulated 25 sets of environmental conditions within each of two ranges of fluctuation. Within each set of environmental conditions we observed the effect of 99 levels of superimposed mortality on up to 50 generations of the same initial population.

At low levels of mortality (1–35%) and the higher level of environmental variation, the predicted size of the spawning population occasionally was far in excess of 60,000 females. Because survival in the Ricker model is an exponentially decreasing function of population size, such large spawning populations produce fewer progeny than the extinction level of 500. If the estimates for α and β obtained in this study were found to hold true for populations whose densities were in excess of the maximum of 60,000 females used in this study, then those extinctions occurring at mortality percentages of 1–35% and a high level of environmental variation could be attributed to overspawning. Since we have no estimates of α and β based upon high population densities, we feel the results are attributable to an overextension of the model and therefore have chosen to disregard

those extinctions occurring at levels of mortality from 1 to 35%.

Figure 4 shows the results obtained from four simulations. In one simulation, the replacement spawning density was exposed to a moderate level of superimposed mortality (50% of the number of females) and a low level of environmental variation. In a second simulation, the same population density was exposed to a superimposed mortality of 50% and a higher level of environmental variation. The third simulation shows the results obtained from the exposure of the population to a high level of superimposed mortality (81%) and a low level of environmental variation. The fourth simulation shows the effects of a high level of environmental variation and a high level of superimposed mortality (81%). In two of four simulations the populations went to extinction within the 50 generations due to the high level of superimposed mortality. A comparison of Fig. 3 and 4 shows that superimposed mortality at a level of 0.50 dampens fluctuations in the population.

Table 3 shows the results obtained from 25 simulations in which environmental variation was within 1 and 2 SD of the mean error. The series

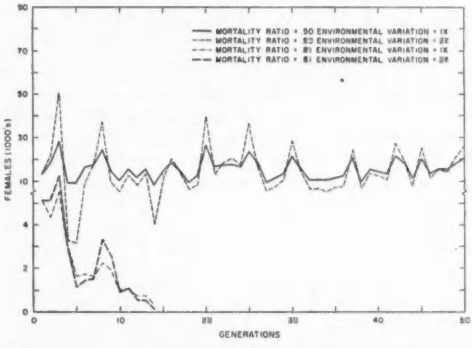

FIG. 4. Time path of the number of females for four simulated populations of fathead minnows, in which the additional effect of superimposed mortality has been added. One simulation shows the effect of a moderate level of superimposed mortality and environmental variation equal to ±1 SD of the mean error term. The second simulation shows the effects of a moderate level of superimposed mortality and environmental variation equal to ±2 SD of the mean error term. The third simulation shows the effects of a high level of superimposed mortality and environmental variation equal to ±1 times the standard deviation of the mean error term. The fourth simulation shows the effect of a high level of superimposed mortality and environmental variation equal to ±2 times the standard deviation of the mean error term.

TABLE 3. Number of extinctions and range of percentage mortalities at which extinctions occurred for two levels of environmental variation. See text for explanation of table.

Random sequence	1×SD	2×SD	Total
1	20(80–99)	22(78–99)	42
2	20(80–99)	21(79–99)	41
3	20(80–99)	21(79–99)	41
4	20(80–99)	23(77–99)	43
5	21(79–99)	24(76–99)	45
6	21(79–99)	23(77–99)	44
7	20(80–99)	23(77–99)	43
8	21(79–99)	25(75–99)	46
9	21(79–99)	23(77–99)	44
10	21(79–99)	25(75–99)	46
11	21(79–99)	26(74–99)	47
12	20(80–99)	22(78–99)	42
13	21(79–99)	24(76–99)	45
14	20(80–99)	23(77–99)	43
15	20(80–99)	23(77–99)	43
16	21(79–99)	24(76–99)	45
17	22(78–99)	29(71–99)	51
18	21(79–99)	27(73–99)	48
19	20(80–99)	25(75–99)	45
20	21(79–99)	26(74–99)	47
21	20(80–99)	22(78–99)	42
22	21(79–99)	26(74–99)	47
23	20(80–99)	21(79–99)	41
24	20(80–99)	24(76–99)	44
25	20(80–99)	22(78–99)	42
Total	513	594	
Mean	20.5	23.8	

of random numbers used in any given sequence was the same for both, once and twice the standard deviation of the mean error. The entries in the body of the table represent the number of times the simulated populations went to extinction as the percentage of superimposed mortality was increased in unit increments from 1 to 99%. The range of percentage mortalities in which the population went to extinction is given in parentheses. For example, for random sequence 10 and environmental variation equal to twice the standard deviation of the mean error, as the superimposed mortality was increased from 1 to 99%, the number of simulated populations that went to extinction before 50 generations had been reached was 25.

The number of extinctions increased substantially with a doubling of the environmental variation but did not differ appreciably from one random sequence to another. There should be no significant difference between random sequences if they are truly random.

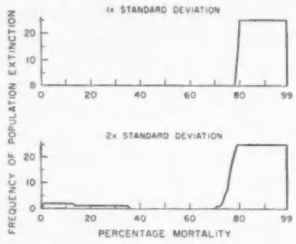

FIG. 5. Histograms of the frequency of population extinction as related to the percentage mortality and simulated environmental fluctuation.

Figure 5 shows the frequency of population extinction as related to the percentage of superimposed mortality for both levels of environmental variation tested. These graphs show that the population first goes to extinction after the superimposed mortality reaches 78% for the lower level of environmental variation and 71% for the higher level of environmental variation. The mechanism responsible for extinctions occurring at mortalities from 1 to 35% was explained earlier and is probably a result of an overextension of the model.

Conclusions

These results show that certain combinations of environmental conditions and superimposed mortalities do not drive the population to extinction if the Ricker model used in this paper is in fact the correct model. To apply these results to a specific pollutant, it appears that an application factor of $\frac{1}{100}$ the 96-hr TL_m value for zinc would, under most conditions, not result in the extinction of fathead minnow populations. This study also shows that waste concentrations of zinc would have to be very carefully controlled to allow for only a 50% reduction in the number of eggs per female. If the egg reduction increased from 50 to 79%, the population would probably become extinct; if the environmental variability doubled, the population would probably become extinct at a superimposed mortality above 71%.

These results also underline the importance of environmental variation as stressed by Brungs (1969); studies of the type he proposed relating to environmental variables will give quantitative answers about the degree of protection afforded to fish populations by application factors. Computer simulations of populations of several species should be used to obtain additional insight into biological effects of varying concentrations of pollutants. The use of simulation techniques that include stochastic processes, as used in this study, will give the biologist the means to include environmental fluctuations in his experimentation.

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Calorie-to-Protein Ratio for Brook Trout (*Salvelinus fontinalis*)[1]

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Based upon a kilogram of feed, the results suggested that the brook trout (*Salvelinus fontinalis*) required approximately 75 kcal of metabolizable energy for each 1% of protein in the feed. Additional feed energy, resulting in greater calorie-to-protein ratios, reduced weight gained but did not significantly increase fat content of the dry carcass.

The carbohydrate and protein of wheat middlings were utilized by the trout. A toasted cereal product composed of 80% corn and 20% wheat was metabolized for energy. Delactosed dried whey was found less effective than glucose as an energy source for trout.

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SALMONOID fishes require diets high in protein. To insure that a substantial amount of protein is not metabolized for energy, it is necessary to know the energy requirement of the various salmonoid species. Knowledge of the energy requirement at a given protein level would establish a calorie-to-protein ratio that would provide proper balance between the energy and protein in a trout feed. Calorie-to-protein ratio would have application to feed formulation when energy content or protein level is changed. Water temperature influences energy and protein requirements of trout, hence calorie-to-protein ratio would have application for different water temperatures. Therefore, the energy requirement and calorie-to-protein ratio for the brook trout (*Salvelinus fontinalis*) was investigated.

Phillips et al. (1948, 1964, 1965, 1966, 1967, 1969) and Poston (1969) have given attention to energy sources and energy and protein utilization by trout. In general, they found that trout utilize corn oil, feeding oil, cottonseed and hydrogenated cottonseed oil, dextrin, and the disaccharide, maltose. With certain diets the protein content could be reduced without loss of growth, provided dietary energy was maintained by addition of fat or carbohydrate. The isocaloric substitution of fat for protein in the diets studied increased body fat. Accumulation of body fat was not prevented by supplemental niacin, choline, or methionine.

Procedure

Two experiments were conducted. Relatively low protein diets, utilizing high quality protein sources, were fed to minimize metabolism of protein for energy. To reduce diet variability and maintain protein constant, all feedstuffs were analyzed for protein before formulation of the diets fed. Energy content of the diets was calculated using the metabolizable energy values of the feedstuffs as determined with poultry (Matterson et al. 1965). The feeds were processed with a laboratory pellet mill into 3.2-mm diam pellets of suitable length for feeding.

The composition of the basic diets is shown in Table 1. Diets 80–89 were fed in experiment 1, and diets 90–94 in experiment 2. Diets 80 and 86 in experiment 1 contained the least and the most energy, respectively; the difference was achieved mainly by replacement of cellulose with glucose. However, it was necessary to add a small amount of corn oil to attain the highest energy level in diet 86. By mixing appropriate proportions of diets 80 and 86, a series of seven isonitrogenous diets varying in energy content was obtained. Facilities were available for study of three additional diets, thus three feedstuffs were evaluated for available energy for trout (diets 87, 88, 89).

Wheat middlings was the test feedstuff in diet 87. Early trout nutrition literature suggested that utilization of the raw starches of cereals and their by-products was low. However, wheat middlings has been a common ingredient of trout feeds, at relatively high levels, for many years. Thus it appeared desirable to evaluate wheat middlings as an energy source.

If cereal starches are not well utilized by trout, it is possible that heat-treated cereals might be utilized better, since Phillips et al. (1948, 1966) have demonstrated the utilization of cooked corn starch and dextrin by trout. Dietary imbalance, particularly in relation to water temperature, may have been a factor contributing to poor carbohydrate utilization in the early research by

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TABLE 1. Composition (percentage) of diet fed brook trout.

Diet no.:	80	86	87	88	89	90	94
White fish meal	33.0	33.0	33.0	33.0	33.0	30.0[a]	30.0[a]
Corn distillers solubles	18.0	18.0	18.0	18.0	18.0	20.0	20.0
Crude casein	5.0	5.0	–	1.25	2.95	1.6	1.6
Dicalcium phosphate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Trace mineral mix[b]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mix[c]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride (25%)	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Cellulose	38.0	–	14.8	17.75	25.05	40.9	11.0
Glucose monohydrate	–	37.5	2.7	–	–	1.5	31.4
Corn oil	–	0.5	–	–	–	–	–
Wheat middlings	–	–	25.5	–	–	–	–
Toasted cereal	–	–	–	24.0	–	–	–
Delactosed whey	–	–	–	–	15.0	–	–
Protein	32.0	32.0	32.0	32.0	32.0	28.0	28.0
Energy, *kcal/kg*	1690	2957	2113	2339	1905	1606	2587
c/p ratio	52.8	92.4	66.0	73.1	59.5	57.4	92.4

[a]Herring meal.
[b]Supplied, in mg/100 g of feed: manganese 15; iodine 0.3; iron 2; copper 0.2; zinc 2; cobalt 0.02.
[c]Supplied per 100 g of feed: Vitamin A palmitate 1000 I.U.; vitamin D_3 90 I.C.U.; vitamin E 2 I.U.; menadione sodium bisulfite 2; thiamine hydrochloride 2; niacin 35; riboflavin 6; calcium pantothenate 10; cyanocobalamin 0.004; pyridoxine 2; folic acid 1; biotin 0.1; *p*-aminobenzoic acid 20; inositol 24; ascorbic acid 25; butylated hydroxytoluene 25 mg.

Phillips. For this reason, a toasted cereal product, composed of 80% corn and 20% wheat, was used in diet 88. This product was heat-treated with 2.11 kg/cm² steam pressure for 35 min. The toasted cereal was a commercial product used in the manufacture of dog food. Since a metabolizable energy value for this product was not available, a value was calculated.

Dried whey represents a potential energy source for trout because of its high lactose content. Therefore, it was used in diet 89.

For experiment 2, protein content of the diets was lowered to 28% to ensure that all protein was utilized for tissue formation when adequate energy was available. By mixing appropriate proportions of diet 90, containing the least energy, and diet 94, containing the most energy, a series of five isonitrogenous diets varying only in energy content was obtained.

In experiment 1, each of the 10 diets was fed to three groups of brook trout in a completely randomized experimental design. At the start of the experiment, each of the 30 groups contained approximately 211 fingerling trout, which averaged 7.6 cm and 5.9 g per fish. The water temperature, 14.5 C at the beginning of the experiment, rose to 17.2 and then fell gradually to 6.1 C in late November at the end of the 20-week experiment.

Experiment 2 was conducted 1 year after experiment 1, using the same facilities and management procedures. Each of the five diets was fed to three replicate groups of brook trout in a randomized block experimental design. Each of the 15 groups contained approximately 207 trout, averaging 6.4 cm and 3.3 g, at the start of the 20-week experiment. Water temperature was 12.8 C at the start of the experiment, rose to 16.7 and then fell gradually to 10 C at the end of the experiment.

The trout were maintained in outside raceways as described previously (Ringrose 1970). Feeding rate was 65% of the Deuel (1952) standard feeding guide. The fish were weighed and counted every 4 weeks. The weight of fish per group and the amount of feed to be fed were adjusted as necessary at these times. Records of weight gain, feed consumption, and mortality were maintained.

At the termination of each experiment two fish were sampled from each group, placed in a moisture-proof bag, and frozen for later analysis. For analysis, each two-fish sample was homogenized, sampled, and triplicate determinations made for moisture, protein, and fat. Analysis of variance and Duncan's multiple range test were used to evaluate the significance ($p < .05$) of the differences among the means obtained.

Results

As shown in Table 2, energy level in the feed and the resulting calorie-to-protein (c/p)[2] ratio affected growth of the trout fed diet series 80–86.

[2]Kilocalories for each 1% crude protein. For clarity, rounded values are used in the text.

TABLE 2. Mean results from feeding diets with varying calorie/protein ratios and testing three feedstuffs as energy sources for brook trout. Experiment 1.

Diet no.:	80	81	82	83	84	85	86	87[a]	88[b]	89[c]
Energy, *kcal/kg:*	1690	1901	2112	2323	2534	2746	2957	2113	2339	1905
c/p ratio:	52.8	59.4	66.0	72.6	79.2	85.8	92.4	66.0	73.1	59.4
Weight gain, *g*	58.2	71.3	78.8	75.0	79.2	73.5	61.9	78.6	78.2	64.6
Feed/gain ratio	1.54	1.35	1.34	1.31	1.28	1.35	1.57	1.36	1.32	1.25
Weighted mortality, %	0.07	0.22	0	0	0.03	0.14	0.21	0.04	0.07	0
Carcass composition (%)										
Wet										
Water	70.8	72.2	72.4	72.0	71.2	70.6	71.4	73.2	72.8	72.9
Protein	16.3	15.6	15.9	16.5	15.6	15.9	15.3	16.7	16.8	16.5
Fat	3.4	4.9	4.4	5.6	6.0	6.8	6.2	3.9	6.1	4.1
Dry										
Protein	57.7	56.2	57.5	58.9	54.8	54.1	53.6	62.2	61.8	60.9
Fat	11.7	17.7	16.1	20.1	20.8	23.2	21.6	14.4	22.4	15.1

[a]Wheat middlings.
[b]Toasted cereal.
[c]Delactosed whey (50% lactose).

Growth with diet 82 was significantly better than with diet 81, thus suggesting a need for a c/p ratio of 66 or more. Growth from diet 85 was not significantly different from diet 84, although it closely approached statistical significance. Hence a c/p ratio within the range from 66 to 79 appears most conducive for growth.

The lowest and the highest energy diets fed resulted in poorer feed conversion than all intermediate diets. There were no significant differences in feed conversion among the other diets in this energy series. However, there was a tendency for c/p ratios 73 and 79 to improve feed utilization. When energy and protein were reasonably well balanced, utilization of the feeds was excellent. Mortality for all diets was low and appeared not to have been associated with any specific diet.

Carcass composition is presented on both a wet and dry basis. Variability in the data is evident but not excessive considering the problems of sampling and preparation for analysis of these large fish. On a dry carcass basis there was no significant difference in protein content of trout fed any of the diets 80–86 in the energy series, nor any of the diets in the experiment. From the lowest energy diet fed, dry carcass fat content appeared to increase up to a c/p ratio of 73, although the difference in fat content between diets with c/p ratios of 66 and 73 was not significant in this experiment. Feeding diets with a c/p ratio of 73 or above did not significantly alter dry carcass fat content.

Diet 87, with a c/p ratio of 66, was formulated to evaluate wheat middlings as a source of energy for trout. The middlings supplied approximately 24% of the total energy in this feed. Comparison of diet 87 with the equivalent c/p ratio diet 82, in which glucose supplied 20% of the energy, showed that the two feeds performed in a similar manner. The differences in dry carcass protein and fat content were not significant. Thus, the energy in wheat middlings was utilized by the trout. Moreover, the metabolizable energy value for poultry assigned to wheat middlings appeared to be fully realized with trout. Whether middlings may have a higher value for trout than for poultry could not be determined from this experiment. The wheat middlings replaced 5% of casein, a high quality protein, in diet 82, and 4.3% of casein protein in the feed. So far as the author is aware, this is the first direct evidence for the utilization of wheat middlings for growth of trout.

Diet 88 contained a toasted cereal (corn and wheat) product as a source of energy. Comparison of diet 88, with a c/p ratio of 73, with equivalent c/p ratio diet 83, showed that the two feeds performed in a similar manner. Thus the energy of the toasted cereal was utilized at least to the extent of the calculated value used in the diet formulation. The toasted cereal supplied approximately 34% of the total energy in diet 88.

Diet 89 contained delactosed dried whey (50% lactose) to supply approximately 15% of the energy in this feed. Upon comparison with diet 81, with a c/p ratio of 59, the trout fed diet 89 gained significantly less weight, although otherwise the diets performed similarly. Apparently, the energy from whey was not as well utilized by trout as by poultry. This probably is due to the

fact that one-half of the lactose molecule is composed of galactose, which Buhler and Halver (1961) have shown is not well utilized by chinook salmon.

The results from the second experiment are tabulated in Table 3. Energy level in the feed and the resulting c/p ratio again significantly influenced growth of the trout. Up to c/p ratio 75, each increment increase in energy in the feed resulted in a significant increase in weight gained. Each increase in feed energy resulting in a c/p ratio above 75 gave a significant decrease in gain when compared to c/p 75. Thus a c/p ratio of 75 was the most effective for growth of the trout. The feed conversion for diets with c/p ratios of 66, 75, and 84 were not significantly different, although the feed conversion for ratio 75 showed some improvement when compared with the other two diets.

On a dry carcass basis there was no significant difference in protein content of the trout fed any of these five diets. In general, fat content of the dry carcass appeared to increase as more energy was fed. However, statistical analysis revealed no significant difference in the fat content of the trout fed diets with c/p ratios of 75, 84, and 92. Sampling may explain the similar fat percentages of trout fed diets with c/p ratios of 84 and 92. Feeding diets with lower c/p ratios of 57 or 66 resulted in significantly less carcass fat than diets with c/p ratios of 84 or 92.

Protein and calorie efficiency data for the better diets in both experiments are shown in Table 4. When protein and energy were reasonably well balanced, protein utilization was very efficient. The trout fed 28% protein with a c/p ratio of 75 consumed only 0.364 g of protein/g of gain. This is the best protein efficiency figure to come to the author's attention for trout averaging 13.7 fish/kg. Caloric efficiency for c/p ratio 75 also was very good. However, calories consumed per gram of gain was influenced by the way the feeds were formulated. Upon consuming an equal amount of feed, or of protein, the trout fed the higher energy diets were forced to consume more calories than the trout fed the lower energy diets.

Discussion

From the data presented it appears clear that a a proper balance between energy and protein is important for optimum growth and feed efficiency of trout, as is true for other species. The data suggest a c/p ratio of 75 as approximately optimum. To what extent one can move in either direction from this ratio is not clear. The first experiment showed essentially the same growth with ratios of 66, 73, or 79, whereas the second experiment resulted in significantly less growth with ratios of 66 or 84 as compared with 75.

Although there were differences in dry carcass protein content these differences proved nonsignificant. This was to be expected since the diets fed within an experiment were isonitrogenous. Also, tissue protein content is quite characteristic of a species and not readily subject to change.

There were some differences in dry carcass fat content. However, there was no significant increase in carcass fat content with c/p ratios above 73 in the first experiment or above c/p ratio 75 in the second experiment. No explanation is apparent as to why carcass fat did not increase with these higher energy diets. Based upon feed consumption and diet composition, the fish fed the highest energy diet, 86, consumed 26% more calories than the fish fed diet 83 with a c/p of 73 in experiment 1.

TABLE 3. Mean results from feeding diets with varying energy contents and calorie/protein ratios. Experiment 2.

Diet no.:	90	91	92	93	94
Energy, *kcal/kg:*	1606	1852	2097	2343	2587
c/p ratio:	57.4	66.2	74.9	83.7	92.4
Weight gain, *g*	53.7	66.4	72.8	65.4	55.4
Feed/gain ratio	1.54	1.35	1.30	1.35	1.56
Weighted mortality, %	0.10	0.07	0.07	0.16	0.13
Carcass composition (%)					
Wet					
Water	73.4	71.8	71.2	71.3	71.1
Protein	17.3	17.3	17.0	16.2	16.5
Fat	3.7	4.2	5.1	6.5	6.5
Dry					
Protein	65.0	61.4	59.0	56.4	57.1
Fat	13.9	14.9	17.7	22.6	22.5

TABLE 4. Protein and calorie efficiency of trout feeds.

c/p ratio	Diet energy (*kcal/kg*)	Consumed/g gain: Protein (*g*)	Consumed/g gain: Energy (*kcal*)	No. fish/kg
		32% protein		
66.0	2112	0.428	2.84	12.7
72.6	2323	0.418	3.05	13.3
79.2	2534	0.410	3.25	12.6
		28% protein		
66.2	1852	0.377	2.50	15.1
74.9	2097	0.364	2.73	13.7
83.7	2343	0.377	3.17	15.3

In experiment 2 the increase in calorie intake for the highest energy diet was 13% when compared with diet 92 with a c/p ratio of 75. The higher energy diets resulted in a dry carcass fat percentage of approximately the same magnitude in both experiments, suggesting the possibility of an upper limit on fat accumulation. Since fat was not accumulated, the surplus energy must have been dissipated to the environment.

A question is raised as to how much fat is desirable? Although there is presently no definite answer to this question, it does appear that in trout for "stocking" a reasonably high fat reserve would be desirable, in view of a period of acclimatization and the uncertainties of a natural food supply.

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Chronic Effects of Low Dissolved Oxygen Concentrations on the Fathead Minnow (*Pimephales promelas*)

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Fathead minnows were exposed to constant dissolved oxygen concentrations (1.0–5.0 mg/liter) for 11 months. The number of eggs produced per female was reduced at 2.0 mg/liter; no spawning occurred at 1.0 mg/liter. Fry growth was reduced significantly at all concentrations below the control (7.9 mg/liter). Fry survival was reduced at 4.0 mg/liter and lower dissolved oxygen concentrations; 18% of the survivors at 4.0 mg/liter were deformed. The time required for hatching was increased at successively lower oxygen concentrations by as much as 50% (from 5.0 under control conditions to 7.8 days at 2.0 mg/liter), but no effect on percentage hatch was observed.

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THE importance of adequate dissolved oxygen in aquatic environments cannot be exaggerated. Oxygen-demanding wastes result from a wide variety of sources and are more widespread than any other principal group of pollutants. Even the less populated areas of the United States are not immune to wastes from paper mills, animal feedlots, food-processing plants, slaughterhouses, etc. A summary of fish kills in the United States (U.S. Department of the Interior 1968) indicates that more than 50% of the fish killed by pollution reported from 1960 to 1968 died as the result of oxygen-demanding, nontoxic wastes. The sublethal effects of reduced oxygen cannot be estimated.

In a recent review of the literature, Doudoroff and Shumway (1970) indicated that no data were available on the chronic effect of low dissolved oxygen concentrations on fecundity and related biological processes.

A chronic exposure of fathead minnows (*Pimephales promelas* Rafinesque) for 11 months to continuous reduced levels of dissolved oxygen was begun in December 1966; reproduction, fry growth, and fry survival were evaluated.

Methods

PHYSICAL AND ANALYTICAL

The nominal experimental dissolved oxygen concentrations were 1.0, 2.0, 3.0, 4.0, and 5.0 mg/liter, and these were controlled ±5–10% (one standard deviation). The control dissolved oxygen concentration was allowed to vary from 5.9 to 9.9 mg/liter (mean = 7.9) as the test temperature varied from 15 to 25 C.

The experimental water was a mixture of pond water originating from a spring at the Newtown Fish Farm (an Ohio Division of Wildlife hatchery, Newtown, Ohio) and carbon-filtered, demineralized tap water. The resulting mixture had a total hardness of approximately 200 mg/liter (as $CaCO_3$) and was stored in an open 400-kliter concrete reservoir.

Water flowed by gravity from the reservoir to a vacuum degassing system (Mount 1961, 1964) in which agitated water was exposed to controlled vacuum levels. A water pump circulated this water through the vacuum chamber and provided a constant flow of water of minimum dissolved oxygen content to a reaeration system. Fry (1951) described a fractionating column to provide water of different oxygen concentrations by removing water from different levels in a vertical column through which a countercurrent of nitrogen was bubbled. This concept was reversed in the reaeration system used (Fig. 1). Water with a dissolved oxygen concentration less than 1.0 mg/liter entered the reaerator at the top and cascaded over a series of removable microscope slides. Water for the nominal concentrations was removed at different points in the reaerator; control water was removed from the low end of the reaerator. Aeration by compressed air increased the oxygen concentration in the control water to near saturation. As the temperature varied, or when the flow rate to each chamber was changed (150 ml/min for the first 4 months, 300 ml/min for the remainder of the test), microscope slides could be relocated, removed, or added. In addition, a different withdrawal point of the reaerator could be used. This system functioned for 11 months with only one malfunction caused by water supply failure to the degasser. The most troublesome problem concerned the water lines leading

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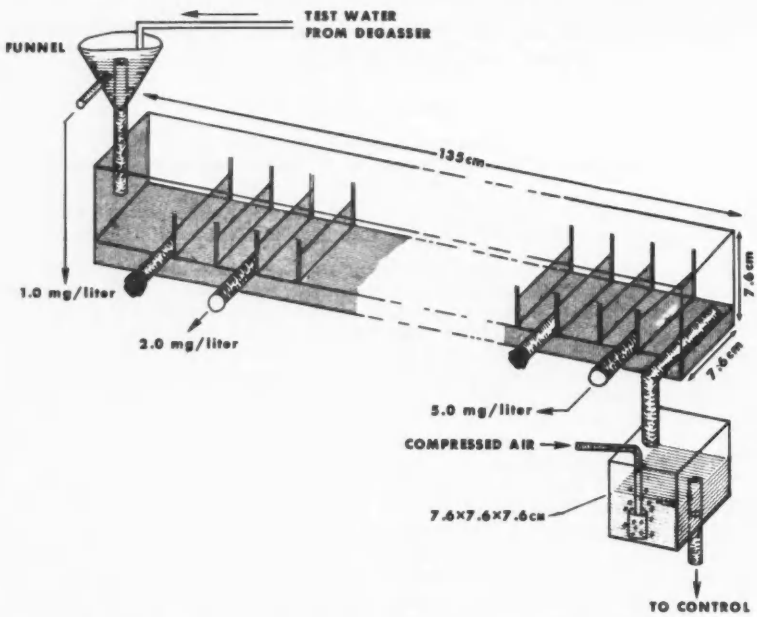

FIG. 1. Reaeration system used to obtain water of various constant dissolved oxygen concentrations for experimental chambers.

from the reaerator to the test chambers: potential flow-rate variation because of detritus necessitated frequent, almost daily, cleaning and maintenance.

The test chambers were made of double strength window glass and measured 30 × 30 × 90 cm; a glass divider provided a 30 × 30 × 60-cm area for the adult fish and a 30 × 30 × 30-cm area for the fry survival and growth studies. The water level was maintained at 20 cm. Water from the reaerator entered at one end of the test chamber through the adult spawning section, flowed into the fry section, and then to waste. Approximately one-half of the waste water was returned to the water supply headbox for the degasser, where it was mixed with fresh water and compressed air. The minimum average daily flow of fresh water to each 45-liter chamber was 220 liters. Glass covers were floated on the water surfaces of both sections of the test chambers with styrofoam to control more adequately the dissolved oxygen concentrations. Small open surface areas were provided for the fish to surface if necessary, as they would in a natural environment. Fish surfaced only at 1.0 mg/liter and only for the first few weeks.

The hatchability tests were conducted with eggs placed in 15-cm glass cylinders (5-cm diam) with fine mesh nylon screen attached at one end suspended in the adult section of the test chambers. These hatching cylinders were attached to an oscillating rocker arm that raised and lowered the cylinders approximately 3 cm several times per minute. Dissolved oxygen concentrations inside the cylinders did not vary by more than 5% from that outside.

The test system was illuminated at a constant 16-hr photoperiod with white fluorescent lights.

Total hardness, conductivity, acidity, alkalinity, and *p*H (Table 1) were determined weekly and dissolved oxygen at least daily according to standard procedures described by the American Public Health Association (1965). Dissolved oxygen concentrations were measured by the sodium azide modification of the Winkler method. Increased accuracy over the standard method was obtained by adding reagents and a magnetic stirring bar, removing 20 ml from each 128-ml sample bottle, and titrating directly into the sample bottle. Nominal concentrations will be used for simplicity in subsequent discussion.

Dissolved oxygen concentrations were measured continuously from time to time to determine the amount of daily variation; the concentrations diminished by 5–10% shortly after the lights were turned off by the timer, and they increased by the same amount just after the lights were turned on again. Sufficient phytoplankton and periphyton were present to cause this diurnal pulse. Daily analyses of dissolved oxygen were made several hours after the concentrations reached the daylight levels.

Temperature was measured daily with a mercury thermometer in two of the test containers. Temperature was 15–18 C during the winter, and it rose during

TABLE 1. Chemical characteristics of the experimental water.

Characteristic	Unit	No. analyses	Mean[a]	Range
*p*H	–	95	7.8±0.1	7.5–8.0
Total hardness	mg/liter as $CaCO_3$	22	212±10	190–224
Alkalinity	"	96	161±8.4	146–178
Acidity	"	93	8.6±2.0	3–14
Conductivity	micromhos	94	444±45	369–508

[a]Mean ± one standard deviation.

the spring so that the usual temperature during the summer spawning season was 23–25 C. Spawning began in April 1967, when the temperature reached 22 C. Recirculation of water in the degasser by the pump raised the temperature of the test water sufficiently to require chilling during the summer months; water being pumped from the degasser to the reaerator was passed through a stainless steel coil submerged in a chilled water bath.

Fathead minnow fry were collected from hatchery ponds in October 1966 and treated from time to time as needed with 40 mg/liter neomycin, 40 mg/liter tetracycline hydrochloride, 25 mg/liter of the fungicide Dexon,[1] and methylene blue before and after the test began.

Twenty 1- to 2-month-old fry were placed randomly in each of the six test containers. Territorial and spawning sites were provided by placing six 7.6 × 7.6-cm half-tiles made of cement and asbestos in each test container. After spawning began, males in excess of the number of spawning tiles were discarded. Typically, the test fish spawned on the ceilings of the tiles, after which the female left the area of the tile while the male guarded the eggs. Several times eggs were not removed from the tiles at the lower dissolved oxygen concentrations; the males guarded these eggs in the usual manner through development. In most instances the monolayer batches of eggs were removed when spawning was completed. Eggs were separated and counted. Approximately 20–30 samples of 50 eggs each were taken from each test concentration over the entire spawning season for hatchability studies. These eggs were 6–8 hr old when they were placed in the hatching cylinders that were suspended in the test container where the eggs were produced. Consequently, hatchability was determined for 1000–1500 eggs from each test concentration over a 3–4-month period. Attempts were also made to hatch control eggs at 1.0 mg/liter.

Fish were fed a dry trout food without antibiotics; feeding was supplemented weekly with cladocerans or small oligochaetes. The fry in the growth and survival studies were fed ad libitum powdered fry food and portions of various microinvertebrate and phytoplankton cultures. The fry studies were conducted for 30 days including the time required for hatching. All chambers were siphoned several times a week to remove uneaten food, excreta, etc., to maintain close to nominal oxygen concentrations. Analyses of these concentrations were made several times just before and 10–15 min after feeding, and no differences were detected. This was true also on the continuous oxygen recordings.

Fry survival and growth studies were begun in August 1967. Fry were placed in the fry section of each test container just after hatching. The only transfer of fry occurred when some produced in the control containers were reared in 2.0 mg/liter of dissolved oxygen.

At the end of the 11-month study in November 1967, the original fish were removed and preserved. Length and weight were determined later.

Results

There was no apparent net effect on reproduction (eggs per female) at concentrations of 3.0 mg/liter and higher (Table 2). The number of eggs per female was lower at 2.0 mg/liter than at higher concentrations, and no eggs were laid at 1.0 mg/liter. The percentage hatch (81–89%) of eggs produced and reared at the same oxygen concentration was not affected where spawning occurred. Control eggs when transferred within a few hours after fertilization to 1.0 mg/liter developed abnormally and died during development. Hatching time varied with temperature, but the average hatching time of eggs increased gradually with decreasing dissolved oxygen concentration from 5.0 to 7.8 days.

Fry-survival data (Table 3) are based on the number of fry added to the fry chambers, not the initial number of eggs used to obtain the fry. Although the percentage hatch of eggs in 2.0 mg/liter was unaffected and the fry produced appeared normal, all of these fry were dead within 6–13 days. Most of them died within 2–5 days. Control fry transferred to 2.0 mg/liter behaved similarly. At 3.0 mg/liter, average survival of fry was only 6% within 30 days after fertilization. Only 24%

[1]Mention of a product does not constitute endorsement.

TABLE 2. Spawning and egg production in the fathead minnow reared from fry at various oxygen concentrations.

Mean measured dissolved oxygen (*mg/liter*)	No. of females	No. of males	spawnings/ female	eggs/ spawning	eggs/ female
1.06	4[a]	3[a]	0	0	0
1.99	5	6	5.4	171	925
3.00	6	4	10.0	176	1763
3.94	4	5	13.3	150	1992
4.91	7	5	16.3	146	2377
7.92 (Control)	8	6	10.6	127	1349

[a]Three of the females and two of the males were immature at the end of the test.

TABLE 3. Survival and growth during 30 days of fathead minnow fry spawned and reared at various dissolved oxygen concentrations.

Mean measured dissolved oxygen (*mg/liter*)	No. replicates	No. fry at start	Survival (%)	Mean length after 30 days[a] (*mm*)[b]
2.02	3	70,38,65	0,0,0	-
3.08	4	70,42,94,91	10,5,3,5	8.7,9.3[c]
4.02	2	70,96	30,20	9.0,9.3
5.01	2	70,73	64,68	9.0,10.3
7.26 (Control)	2	70,70	50,50	12.2,11.1

[a]Thirty-day period includes the time required for hatching.

[b]Mean length of 143 newly hatched fry from a control dissolved oxygen concentration was 5.3 mm.

[c]Only two of the four replicates were continued for 30 days.

survived at 4.0 mg/liter, and of these, 18% were deformed (acute lateral flexion). Fry survival at 5.0 mg/liter and the control was acceptable based on other studies with the fathead minnow at this laboratory, although control fry survival was near the lower level of acceptability.

Growth of the control fry (Table 3) significantly ($\alpha = .01$) exceeded that of fry reared at the lower oxygen concentrations. Fry growth at 5.0, 4.0, and 3.0 mg/liter was not significantly ($\alpha = .01$) different using analysis of variance and the Student-Newman-Keuls procedure (Sokal and Rohlf 1969, and Rohlf and Sokal 1969). Length and weight data for the adult fish at the end of the test cannot be used for comparisons because of the small number of test animals and the size variations within the experimental chambers.

Discussion

Fry growth was the most sensitive indicator of sublethal effects of dissolved oxygen with fry survival nearly as sensitive. Reproduction was the least sensitive. No effect on percentage hatch was indicated except for control eggs transferred to 1.0 mg/liter. Fry growth was reduced significantly at all exposure concentrations below that of the control. The inverse relation between the average number of eggs per spawning and dissolved oxygen concentration may have been the result of less frequent spawning, although in an earlier study, elevated temperature reduced both the frequency of spawning and the average number of eggs per spawning (Brungs 1971). The results of this earlier study showed that reproduction was by far the most limiting life cycle stage for elevated temperature.

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Degradation of Inosine 5′-Monophosphate in the Skeletal Muscle of Several North Atlantic Fishes

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The rates of breakdown of inosine 5′-monophosphate (IMP) and of its product, inosine (Ino), varied considerably in the muscles of Atlantic cod, haddock, pollock, Atlantic halibut, American plaice, and winter flounder when stored in ice. Haddock and Atlantic halibut were noteworthy in having a very slow rate of breakdown of IMP in relation to the other species. Variations of the rate of dephosphorylation of IMP among individual winter flounder appear to be due to differences in muscle *p*H. In homogenates of the muscles investigated, the maximum rates of breakdown of IMP occurred in the *p*H range 8.6–9.4, and were much greater than in the *p*H range of muscle. The effect of *p*H on the breakdown of Ino was less pronounced. In haddock and Atlantic halibut there was little or no loss of endogenous IMP when the muscle was cooked at 260 or 100 C; IMP added to Atlantic cod before cooking was also stable.

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THE finding that inosine 5′-monophosphate (IMP)[1] can contribute to flavor enhancement in meat products has stimulated an interest in its postmortem formation and breakdown in muscle tissues used as food (for history and development see Kuninaka et al. (1964)). Such studies of a number of species of fish in recent years have shown that the rate of breakdown of IMP may vary considerably from one species to another (Saito and Arai 1958; Saito et al. 1959; Jones 1963; Kassemsarn et al. 1963; Fujii et al. 1966; Dyer et al. 1966; Spinelli 1967; Fraser et al. 1967, 1968a, b; Groninger and Spinelli 1968; Kemp and Spinelli 1969). We have confirmed this variation in an examination of the postmortem changes of six adenine derivatives in the muscle of six species of commercial marine fishes from North Atlantic waters, and have found that gross variations were reflected in differing rates of IMP hydrolysis by aqueous homogenates of these muscles under standard conditions. We also report here some preliminary experiments to investigate the effect of cooking upon the IMP already present in some fish muscle. Information on the rate of formation of hypoxanthine in the stored muscle was also needed, since this base is attracting an increasing interest as a quality index.

Experimental

The species studied were; Atlantic cod (*Gadus morhua* Linnaeus 1758); haddock (*Melanogrammus aeglefinus* (Linnaeus) 1758); pollock (*Pollachius virens* (Linnaeus) 1758); American plaice (*Hippoglossoides platessoides* (Fabricius) 1780); winter flounder (*Pseudopleuronectes americanus* (Walbaum) 1792); Atlantic halibut (*Hippoglossus hippoglossus* (Linnaeus) 1758); and redfish (*Sebastes marinus* (Linnaeus) 1758). Except for the redfish, the animals were obtained alive; the redfish used were either post-rigor or frozen. The live fish were held for varying times up to 2 months in seawater aquaria with food available to them. They were stunned by a blow on the head, most of the blood was drained away by severing the tail, and the fillets were removed and skinned. After removal of a small sample for extraction with perchloric acid, one fillet was placed in a polyethylene bag and stored in crushed ice. Further samples for extraction with perchloric acid were taken from time to time from the thick dorsal area of this fillet, avoiding skin and dark muscle, in order to reduce the effect of possible nonuniform distribution of metabolites (Fraser et al. 1966). The second fillet was used in preparation of an extract for measuring enzymic activities.

[1]Abbreviations used: AMP, ADP, ATP, adenosine 5′-mono-, di-, and triphosphate, respectively; IMP, inosine 5′-monophosphate; Ino, inosine; Hx, hypoxanthine; TLC, thin-layer chromatography; Tris, tris (hydroxymethyl) amino methane; EDTA, ethylenediaminetetraacetate.

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Weighed muscle samples of approximately 5 g were blended with 10.0 ml of 6% $HClO_4$, centrifuged for 5–10 min at 40,000 × *g*, then neutralized with saturated KOH. The clear supernates were assayed for nucleotides and degradation products by thin-layer chromatography (TLC) (Randerath and Randerath 1966; Fraser et al. 1967). After development with water and the LiCl system, the separated spots were cut out and eluted with 2 M NaCl. Ultraviolet absorption spectra of the centrifuged eluates were determined, and when the ratio of absorptions at two suitable wavelengths did not agree with that for the pure substance, a correction for variation in background was applied. Molar absorbancy indices used in calculating concentrations were obtained from measurements on the reagents supplied by Schwarz BioResearch Inc., Calbiochem, and Nutritional Biochemicals Corp. The indices at *p*H 7 were 11.5×10^3 at 250.5 nm (the isobestic point) for adenosine and inosine compounds, and 10.3×10^3 at 250.5 nm for hypoxanthine. To facilitate comparisons, results are presented as percentages of total nucleotides and degradation products present in each sample, or of substrate at zero time in the case of enzymic activity assays.

The extract for assay of enzymic activity was made by homogenizing 50 g of muscle with 400 ml of water in a Waring Blendor fitted with a baffle plate to prevent vortex formation (Dyer et al. 1950). Reaction mixtures contained 8.0 ml of homogenate or of supernate from centrifuging the homogenate at 14,000 × *g* for 30 min, 1.0 ml of 15 mM substrate (IMP or Ino, Schwarz BioResearch Inc.), and 1.0 ml of buffer (0.25 M Tris and 0.25 M imidazole, *p*H 8.5–8.7). When the effect of *p*H on the reaction was to be measured, only 5.0 ml of the protein extract was used, and with the same buffer the *p*H was set to the desired value with HCl or NaOH before adding the substrate. In some instances, the homogenate or supernate was dialyzed against two or three changes of about 20 volumes of water in order to reduce the inorganic phosphate content.

The *p*H did not change significantly in the region of 8.5, nor increase by more than 0.14 unit at values below 6 in the course of any reaction. Incubation in all instances was at 30 C by immersion in a water bath. Reactions were stopped by addition of 1.0 ml of 33% (w/v) $HClO_4$, the precipitated protein removed by centrifugation, and the supernates neutralized with saturated KOH (about 0.35 ml). These were analyzed by TLC, or in some instances in which the extracts had been dialyzed, they were assayed for inorganic phosphate (Rockstein and Herron 1951).

Homogenates of 2–3 g of muscle in 10 ml of 0.005 N sodium iodoacetate were used for *p*H determination (Marsh 1952).

Results

Changes in Iced Fillets

The changes in amounts of purine derivatives in the fillets stored at 0 C is shown in Fig. 1. Except for winter flounder, in this preliminary survey only one fish in each species was studied in this way. In one experiment, six winter flounder (one male and five females, ready to spawn) were all killed, filleted, and iced within 30 min. For the sake of clarity, only the changes in IMP and Hx are shown in Fig. 1F for these fish; Ino, when found, did not exceed 5%, and the changes in the other constituents were similar to those shown for the other fishes.

Figure 1 shows that there was considerable variation in the amount of ATP at "zero" time, presumably reflecting differing degrees of exercise of the fishes by struggling before killing. The ATP in all instances fell to less than 10% of the total purines within 2 days. The ADP content, after falling in the first 2 days, thereafter remained fairly constant at 5–10% of the total. This amount probably represent the ADP that comprises a prosthetic group of actin. In no instances were we able to detect any AMP.

The most significant differences between fishes occurred in the rates of production and decay of IMP, Ino, and Hx. In all instances, IMP rose to a maximum between 1 and 2 days on ice, and in Atlantic cod, pollock, American plaice, and four of the winter flounder specimens, fell to a relatively low value after 4 days. However, in haddock, and especially in Atlantic halibut, the decay of IMP was much slower. This was indirectly confirmed when several samples of postrigor haddock and Atlantic halibut, purchased from a retail store for another purpose, were found to contain a high level of IMP. On the other hand, the graphs for the six winter flounder (Fig. 1F) show that considerable variations can occur among fish of the same species. The most aberrant of the group was the male, and it may be noteworthy that in the five female fish, the *p*H increased from a range of 6.5–6.8 at 1 day to 6.7–6.9 between 4 and 9 days, but was considerably lower in the male fish, approximately 6.4 between 4 and 9 days.

Inosine reached a maximum of 60% of the total purine compounds present in Atlantic cod, and 80% in pollock within 4 days, and remained high for up to 10 days. In haddock and Atlantic halibut, Ino rose gradually to intermediate levels and then slowly fell again. In American plaice, Ino reached a maximum of only 10% within 1 day and then fell, whereas in only two out of eight winter flounder could any Ino be detected at any time. The differences among the fishes in Ino content at various times of storage at 0 C resulted from differing rates of breakdown of IMP and of production of Hx. Hypoxanthine was formed only in small amounts in pollock, haddock, and Atlantic cod during 10 days storage, and thereafter its

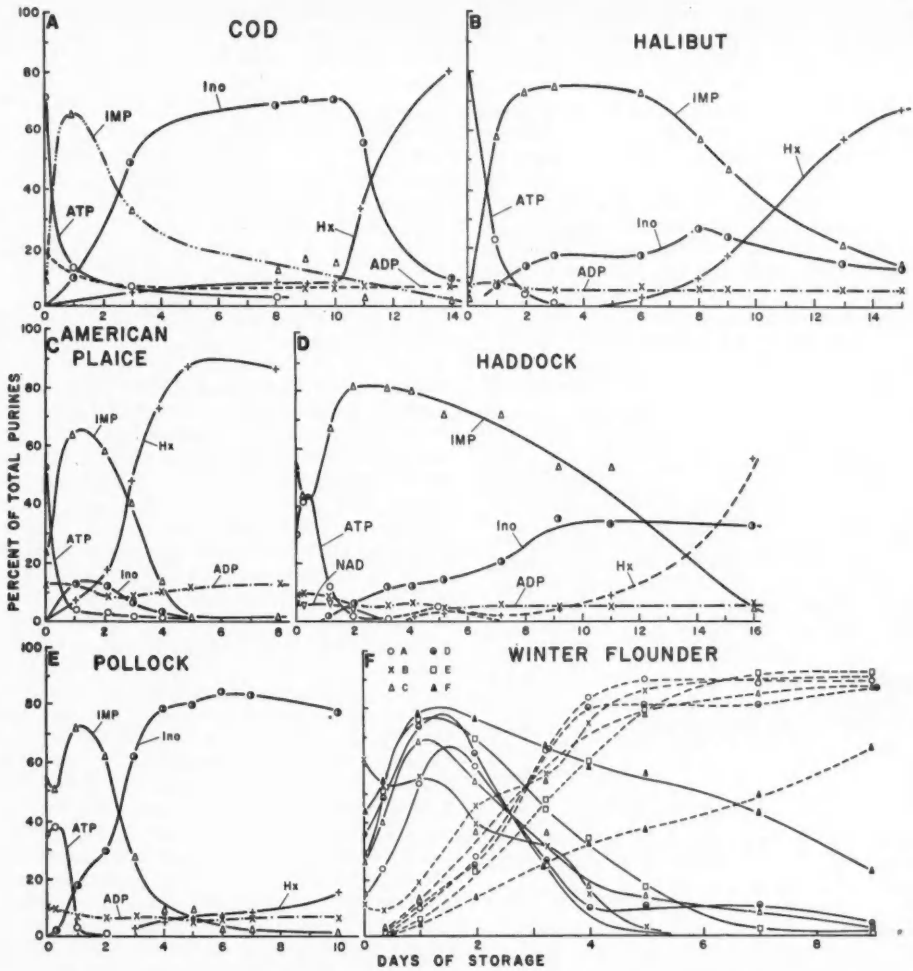

FIG. 1. Changes in composition of purines in perchloric acid extracts of fillets of several commercial fish species stored at 0 C. Ordinates, components as percent of total purines in the extract; abscissae, days of storage. Fig. 1F: changes in IMP (solid lines) and Hx (broken lines) in fillets of a group of six winter flounder stored at 0 C. Coordinates as above.

production might be attributed to bacterial action (Jones et al. 1964). In Atlantic halibut, Hx gradually increased after the 6th day. In American plaice, and most of the winter flounder, however, Hx was formed within 1 day, and rose rapidly thereafter to about 90%. Similar behavior was reported for redfish by Fraser et al. (1968b). In these fishes, therefore, one would expect to find a relatively active Ino–ribohydrolase (EC 3.2.2.2) or phosphorylase (EC 2.4.2.1), or both.

The total purines found in the samples taken at intervals from the fillets of the group of six winter flounder stored at 0 C are given in Table 1. A similar degree of variation in samples was found with the other fishes examined, but the average amounts of purines differed from species to species. These averages, expressed as μmoles per gram of wet muscle were: Atlantic cod, 5.6; American plaice, 5.1; Atlantic halibut, 7.6; haddock, 7.7; and pollock 7.5.

TABLE 1. Total purines in perchloric acid extracts of fillets of winter flounder stored at 0 C. Values are expressed as μmoles per gram of wet muscle, and are the totals of the purines recovered from thin-layer chromatograms.

Fish	Storage time (*days*) at 0 C								
	0	0.4	1	2	3.5	4	5	7	9
A	5.2	4.9	5.1	5.0	4.6	4.5	4.5	4.8	4.7
B	5.3	4.9	4.8	4.4	4.6	4.2	4.1	4.3	4.2
C	4.4	4.5	4.8	5.2	5.3	5.4	5.4	5.9	5.9
D	4.4	4.0	5.6	5.4	4.8	5.1	4.9	5.0	4.9
E	5.1	5.2	5.4	5.9	5.8	5.9	6.1	6.1	5.9
F	4.5	4.1	4.5	4.6	4.9	4.9	4.6	5.1	5.1

REACTIONS IN MUSCLE HOMOGENATES

The effect of *p*H on the IMP-phosphohydrolase activity in extracts from individuals of several species is shown in Fig. 2. The optimum *p*H was in the range 8.6–9.4, in agreement with other nucleotidases (Laskowski 1951).

The occurrence of maximum activity at a *p*H near 9 suggested the possibility that the reaction was catalyzed by a nonspecific alkaline phosphatase. When centrifuged aqueous extracts of Atlantic cod and of frozen redfish, dialyzed to reduce the inorganic phosphate content, were tested with 1.5 mM β-glycerophosphate as substrate, the production of inorganic phosphate was less than 8 and 13% respectively of the corresponding production from 1.5 mM IMP. Guanosine 5′-monophosphate, although closely related to IMP, and also uridine 5′-monophosphate, were poor substrates. Hence, nonspecific alkaline phosphatase, if present, appeared to be only a minor component of the activity.

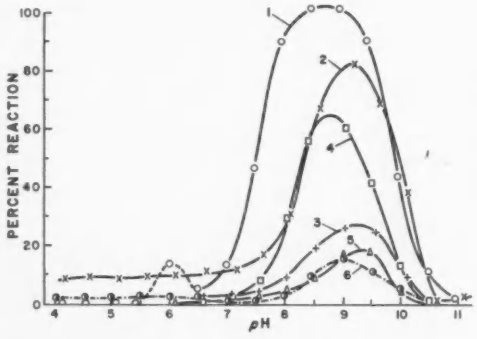

FIG. 2. Effects of *p*H on rate of dephosphorylation of IMP in homogenates of muscle of several commercial fishes. Points plotted are initial rates as percent reaction per hour at 30 C. 1, American plaice (dialyzed); 2, Atlantic cod; 3, Atlantic cod (centrifuged and dialyzed); 4, haddock; 5, Atlantic halibut (centrifuged and dialyzed); 6, winter flounder (centrifuged and dialyzed). Reactions in extracts 2 and 4 were assayed by TLC for IMP, Ino, and Hx; extracts 1, 3, 5, and 6 were assayed by measurement of production of inorganic phosphate.

When KCl was added to reaction mixtures containing a centrifuged and dialyzed extract of frozen redfish muscle, the rate increased slightly up to 0.1 M KCl (from 75 to 87% per hr), and decreased gradually to 60% per hr with further addition of KCl to 0.6 M. Magnesium ion appeared to have no effect, apart from a small one due to its contribution to ionic strength. Nickelous ion reduced the activity of an Atlantic cod homogenate by 30% at 10^{-5} M, and by 90% at 10^{-3} M, which tended to confirm the absence of a nonspecific alkaline phosphatase (Ahmed and Reis 1958). EDTA inhibited the activity completely at 3×10^{-4} M and by 12% at 1×10^{-4} M, possibly as a result of removal of very small amounts of magnesium or other divalent cations frequently required for activity of phosphatases. A similar inhibition was noted by Tarr et al. (1969) with Pacific cod muscle 5′-nucleotidase, and also by Groninger and Spinelli (1968) when fish muscle was treated with EDTA.

The progress curves for the group of six winter flounder studied simultaneously are shown in Fig. 3. There were considerable differences in the rates with the time to 50% loss of IMP varying from 0.8 to 2.0 hr. Moreover, these differences did not agree with the variations found for the decay of IMP from the maximum values attained in the corresponding fillets (Fig. 1F), perhaps because of the varying fillet *p*H already noted (Table 2).

Figure 3 also shows that in the presence of winter flounder muscle homogenate, at *p*H 8.6, Ino is degraded to Hx, but less rapidly than it is formed from the original IMP substrate. Since the resultant

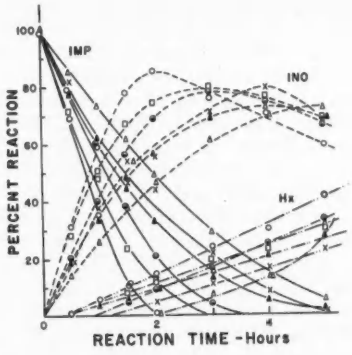

FIG. 3. Rates of dephosphorylation of IMP in homogenates of six winter flounders examined simultaneously; *p*H 8.6, 30 C. Points ○, ×, △, ◒, □, and ▲ are for fish A to F respectively (see Fig. 1F).

TABLE 2. Rates of breakdown of IMP in extracts and in the corresponding fillets of winter flounder. Reactions in extracts were carried out at 30 C and *p*H 8.7; fillets were stored at 0 C.

Fish	Rate (%/*hr*)		Ratio
	Extract	Fillet	
A	65	1.1	58
B	41	0.54?	76
C	28?	0.62	45
D	43	1.3	33
E	58	0.75	77
F	38	0.27	140

temporary but substantial accumulation of Ino did not occur in stored fillets, the relative rates of the two reactions were probably reversed at the lower *p*H of the fillets. The Ino–Hx reaction catalyzed by winter flounder homogenate was, indeed, less dependent on *p*H than was the IMP/Ino reaction (Fig. 4). Tarr (1955) also found a rather broad *p*H range for the activity of whole muscle riboside hydrolase from ling cod and rock cod, although "purified preparations" had two distinct maxima at *p*H 5 and 9. In one experiment with Ino as the original substrate, we followed the formation of Hx by both TLC and the xanthine oxidase assay (Jones et al. 1964, modified from Kalckar 1947); the results of the two procedures yielded indistinguishable rates for the reaction. A strong Ino–ribohydrolase activity was also found in water homogenates of unfrozen and frozen redfish, a species that also exhibits rapid Hx production in iced storage (Fraser et al. 1968b).

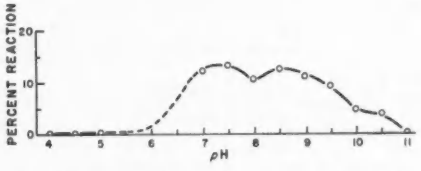

FIG. 4. Effects of *p*H on the rate of formation of Hx from Ino in a homogenate of winter flounder muscle; incubated 5 hr at 30 C.

When a water homogenate of haddock muscle was centrifuged for 30 min at 14,000 × *g*, the clear supernate showed a much lower IMP-phosphohydrolase activity than the homogenate (Fig. 5), so that a large part of the activity in this instance appeared to be associated with cell particles or possibly structural proteins. Homogenates of muscles of Atlantic cod and American plaice also showed a loss of activity upon centrifuging, but less severe. The strong curvature of the reaction line observed with haddock homogenate persisted in the reaction with the supernate. This may indicate loss of activity of the enzyme during the course of the reaction at 30 C, but was not further studied.

EFFECT OF COOKING

The above results suggested that unfrozen Atlantic halibut and haddock obtained from retail sources would probably contain near-maximum amounts of IMP, and the fate of this in cooking would be of interest. Samples of such fish were cooked in two ways: (*a*) about 5 g in 50-ml centrifuge tubes, either stainless steel or polypropylene, were immersed in boiling water for 20 min; (*b*)

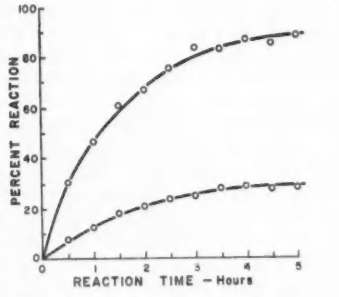

FIG. 5. Rate of dephosphorylation of IMP by an extract of haddock muscle at *p*H 8.5 and 30 C. Top curve, homogenate; bottom curve, supernate after centrifuging the homogenate at 14,000 × *g*.

samples of not less than 50 g, 2–3 cm thick, were baked uncovered on aluminum foil in a kitchen oven for 10 min at 260 C. After cooking, $HClO_4$ extracts of cooked and raw samples were assayed by TLC. No allowances were made for loss of moisture in cooking, but the entire sample in method (*a*), and a sample representative of that which might be used in a serving from method (*b*), were taken for this assay. Assays showed that the fish did, indeed, contain important amounts of IMP (64, 86, and 84% of total purines in halibut samples; 80 and 31% in haddock), and that these proportions were not changed by any of the cooking methods.

Commercial postrigor Atlantic cod fillets examined did not contain any measurable amount of IMP, a finding to be expected from the relatively high IMP-phosphohydrolase activity in this muscle. In two experiments, postrigor Atlantic cod, which contained Ino but no IMP, was passed through a meat chopper, and IMP added as a 0.6 M solution, to a concentration of about 6 μmoles/g. The treated muscle was promptly baked as above, and, according to TLC, only about 10% of the IMP was degraded to Ino.

Discussion

We did not fully realize our hope that the relative activities of IMP-phosphohydrolase in the muscles of various fishes could be conveniently and quickly estimated by an assay of the activities in aqueous extracts under standard conditions. The unknown but apparently important effect of minor changes in *p*H in the physiological range made it impossible to judge whether the extracts represented a true sampling of the activity in the muscle. Among the group of winter flounder, for example, the ratios of the rates in fillets and in extracts showed poor agreement (Table 2), especially in fish F, in which the *p*H of the fillet was lower than the others. Obtaining reliable data on the effect of *p*H in the physiological range will probably require the partial purification and concentration of the enzyme, since at present the reaction times are so long that bacterial activity becomes a problem. Another possible cause of variation in extract activity could be varying amounts of dark muscle tissue included in the sample. The fish used in this work were "white-fleshed," and we avoided, as well as possible, inclusion of the small amounts of dark tissue near the skin, but this was difficult in the case of the smaller flatfish, winter flounder and American plaice. In comparing activities in extracts of different species, there is the added complication that it would be necessary to take into account the differences in *p*H of maximum activity.

It is risky to draw conclusions about enzyme activities in tissues from results obtained with extracts; nevertheless, some speculation might be attempted. In our extracts, the enzyme concentration was probably reduced to one-ninth the apparent concentration in the muscle, but the reaction rate in muscle at 0 C might be expected to be about one-eighth of that in the extracts at 30 C. These two effects should roughly offset each other. The curve for activity vs. *p*H for the Atlantic cod enzyme (Fig. 2) suggests that the rate in the standard reaction mixture might be about eight times that in the stored muscle. It is difficult to estimate from the data the rate of breakdown of IMP in the stored fillets after the precursors were exhausted, but for Atlantic cod this was roughly 20% per day, and for the other fish varied between 7 and 31% per day. This means that the rates of breakdown of IMP in the extracts were between 30 and 140 times (40 times for Atlantic cod) greater than the rates in the corresponding fillets. Thus, it is doubtful that *p*H is the only factor that affects the ratio of rates. The difference between the average tissue *p*H and the optimum for the enzyme in extracts is by no means unusual, and unless the enzyme actually does operate under inefficient conditions in the muscle, it may exist in a microenvironment different from the muscle average. This would not be surprising if in the muscle the enzyme were fixed to a matrix of some kind, and our finding that much of the activity is sedimented by centrifuging would support this suggestion. In this instance the low rate of reaction in muscle might be accounted for by the necessity of diffusion of reactants to the enzyme sites.

Similar considerations probably apply to the differences observed in the production of Hx from Ino. In winter flounder and American plaice this reaction was more rapid than the breakdown of IMP, so that little Ino ever accumulated in these muscles; the reverse was true in the corresponding extracts at *p*H 8.7. On the other hand, Ino accumulated in Atlantic cod and pollock muscle and was not observed to form Hx in the extracts either. These results strongly suggest that rapid Hx formation in the early stages of storage of certain fishes is due to an endogenous enzyme, whereas in others the enzyme appears to be missing. Hx formation in the latter is most probably due to bacterial action.

The results of this work add to the growing body of evidence that the activity of concentration of the Hx-producing enzyme may vary widely in the skeletal muscle of different species of fish. Moreover, the experience with winter flounder shows that the physiological condition of the muscle may significantly affect the production of Hx by

altering the activity of one or more of the enzymes in the purine degradation chain. It is clear that Hx cannot be used as a quality index in the absence of a rather detailed study of the storage behavior of each species of interest.

Although it was interesting to find that IMP persists for several days in the muscle of haddock (unlike its close relative, Atlantic cod) and Atlantic halibut, confirming the reports of Kassemsarn et al. (1963), and of Spinelli (1967), this would have been of little significance to the consumer if it were destroyed in cooking. Happily, this does not appear to be the case (see also Jones 1963), although Spinelli (1967) did find losses in cooking certain Pacific species. On the other hand, even though IMP added to a relatively fast hydrolyzer like Atlantic cod survived after rapid cooking, this technique would have limited household application but might be applied to canned and other precooked products (Hashida et al. 1968).

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Migrations of Adult and Juvenile Walleyes (*Stizostedion vitreum vitreum*) in Southern Lake Huron, Lake St. Clair, Lake Erie, and Connecting Waters[1]

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FERGUSON, R. G., AND A. J. DERKSEN. 1971. Migrations of adult and juvenile walleyes (*Stizostedion vitreum vitreum*) in southern Lake Huron, Lake St. Clair, Lake Erie, and connecting waters. J. Fish. Res. Bd. Canada 28: 1133–1142.

Recoveries of walleyes (*Stizostedion vitreum vitreum*) tagged as adults and juveniles at various sites in waters from southern Lake Huron to eastern Lake Erie provided information on the migrations of the Thames River stock and the mixing of these fish with other stocks in the study area. Walleyes spawning in the Thames River in March and April quickly migrated into the St. Clair River and southern Lake Huron, where they mingled with other stocks, including some from Lake Erie. The return migration to the Thames River occurred between November and March. Juvenile walleyes tagged in Lake St. Clair, on the other hand, moved downriver to Lake Erie. Young-of-the-year fish tagged in western Lake Erie, many presumably of Lake St. Clair origin, provided evidence of a reverse migration, since they tended to move back into Lake St. Clair and Lake Huron as they matured. Adult walleyes tagged along the Canadian shore of Lake Erie migrated eastward during the summer, but were recovered in or near the western basin during the spawning season. Thus, western Lake Erie appeared as a juvenile milling or mixing area during the summer, whereas southern Lake Huron and the north shore of central and eastern Lake Erie were adult areas.

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THE sudden appearance each spring of great numbers of walleyes (*Stizostedion vitreum vitreum*) spawning in the Thames River raised questions as to their distribution the remainder of the year. They were not considered abundant in adjacent Lake St. Clair except during the spring. A tagging programme was therefore initiated to determine their dispersal after leaving the river. Preliminary results from this and another study (Ferguson and Cummins MS 1956) revealed the necessity of expanding the tagging operations to adjacent areas. In addition, early results suggested that juvenile walleyes should be tagged, since it appeared that they must migrate in a manner distinct from that of adults. Between 1955 and 1960, and as opportunity permitted, the study area was expanded to include southern Lake Huron, Lake St. Clair, western Lake Erie, and contiguous waters (Fig. 1). A number of walleye spawning grounds are known to be contained within these areas (Regier et al. 1969). The principal objective of tagging fish in these several locations was to define the major migration patterns for walleyes in these areas. The migrations of walleyes in central and eastern Lake Erie had also been studied by means of tagging from 1954 to 1957, but the results were limited. However, examination revealed that they contributed to our understanding of walleye movements in western Lake Erie. Therefore, the whole of Lake Erie was included in the study area (Fig. 1).

In most previous studies, walleyes have been considered as typically a far-ranging species. Recent investigations by Forney (1963) and Magnin and Beaulieu (1968), however, indicated that walleyes tend to confine their movements to restricted "home" areas throughout most of the year, and that they undertake extensive migrations only prior to and after their spawning season. Their findings agree with the concepts of restricted movements and territorial behaviour of fishes discussed by Gerking (1959). A walleye stock may therefore be identified either by its spawning area or by its "territory." In this study, a stock was

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defined as a group of walleyes associated with a particular spawning area and the movements to this area as "homing."

In discussing walleye movements we have accepted the conclusion that walleyes, rather than indiscriminately spawning in any suitable areas, return to their natal areas. Studies on homing of adult walleyes (Stoudt and Eddy 1939; Olson and Scidmore 1962; Crowe 1962; and others) have shown that most return to the same spawning site in successive years. These studies suggest that discrete spawning stocks of walleyes do exist, and may be considered genetic entities similar to races of Pacific salmon. It has not been shown that walleyes home to natal streams or shoals. There is, however, circumstantial evidence from the work of Rawson (1957) that suggests this does occur. He observed that walleye stocks spawning in two tributaries of Lac La Ronge differed in size at maturity and in fecundity, indicating that they may be distinct races.

There has been much discussion and study concerning the deterioration of environmental conditions in Lake Erie and it has been implied that fish stocks have suffered as a consequence. This subject will not be reviewed here, nor will it be disputed that walleye stocks have in some way been affected. Pollution did not appear to be a factor in the distribution of walleyes in this study. Walleyes moved through all waters from western Lake Erie to Lake Huron. In particular, many walleyes were recovered from parts of western Lake Erie, where pollution has been most severe. It is not known if the movements of walleye stocks have changed significantly since this study, or will change in the future as a consequence of continued pollution.

Methods

The walleyes to be tagged were obtained directly from commercial trapnets, poundnets, gillnets, and seines. They were held in fresh water without anaesthetic, and only those in good condition were tagged. Most of the fish were tagged with plastic streamer tags, which were attached behind the head and anchored through the supraoccipital process with nylon monofilament thread. They were attached snugly to minimize tag flutter and tissue cutting. Examination of recovered fish was possible through the cooperation of sports and commercial fishermen, who not only returned tags and information but frequently turned in the whole fish. Examination of these fish indicated that there was limited enlargement or infection of the wound when snug loops were used to attach the tag, and little suggestion that tag loss might have occurred. A limited number of fish were also tagged using various sizes of monel metal jaw tags.

Jaw tags were more readily overlooked than streamer tags by commercial fishermen and, because these men contributed many recoveries, streamer tags showed a higher recovery rate. However, only a small number of jaw tags were used, and tagging at all times was mainly with streamer tags. Therefore, any real differential in recovery, loss, or mortality due to tag type should have no effect on the results for present purposes.

During 1955 and 1958, a total of 1089 walleyes were tagged in Lake Huron (Table 1). They had been caught in southern Lake Huron within 15 miles northeast of Point Edward. Those fish tagged in 1955 were mostly age III (Table 2); they were released at the capture site (Fig. 1). In 1958 the fish were mainly age II, and were released in the St. Clair River just below Point Edward.

TABLE 1. Numbers and modal ages of walleyes tagged, and percentages recovered, at various locations during the study.

Release area	Date released	Modal age	No. released	% recovered
L. Huron	July 1955	III	381	8.3
L. Huron	Nov. 1958	II	708	19.1
Thames R.	Apr. 1956	V	896	12.6
Thames R.	Mar. 1957	III and VI	1897	16.3
L. St. Clair	May, Oct. 1955	III	458	8.3
L. St. Clair	Feb., Nov. 1960	I only	712	10.4
Detroit R.	Nov. 1958	IV	24	20.4
L. Erie				
Pt. Pelee	Oct. 1959	O only	583	19.7
Wheatley	Summer 1954	II	228	5.2
Port Stanley	Summer 1955	I	232	5.6
Nanticoke	Summer 1956	II	47	12.8
Port Burwell	Sept. 1957	III	74	8.1
Total			6240	13.7

TABLE 2. Percentage age compositions of walleyes released, and their contributions to the recoveries, Thames River, Lake St. Clair, and Lake Huron tagging. (Rel, released; Rec, recovered.)

	Thames R. 1956–57		L. St. Clair 1955		L. Huron 1955		L. Huron 1958	
Age	Rel	Rec	Rel	Rec	Rel	Rec	Rel	Rec
I	–	–	5.2	–	–	–	0.8	–
II	0.1	–	4.4	–	0.3	3.6	57.8	48.5
III	19.1	14.4	56.5	47.4	86.0	82.2	16.7	11.9
IV	16.5	17.9	21.2	18.4	10.5	14.3	37.0	35.8
V	21.8	18.9	8.8	15.8	0.2	–	1.2	0.7
VI	21.0	26.6	1.6	10.5	–	–	1.4	1.5
VII	9.7	11.4	1.0	2.6	–	–	1.2	0.7
VIII	5.3	4.5	1.3	5.3	–	–	–	–
IX	5.3	5.2	–	–	–	–	0.3	0.7
X	0.8	0.5	–	–	–	–	–	–
XI	0.2	0.2	–	–	–	–	–	–
XII	0.1	0.2	–	–	–	–	–	–
XIII	<0.1	–	–	–	–	–	–	–
No. fish	2468	402	386	38	360	28	686	134

FIG. 1. Study area and tagging locations.

Walleyes tagged in Lake St. Clair in 1955 were mostly age III and they were caught and released near the Thames River mouth (Fig. 1). During 1960, most of the yearling walleyes tagged in Lake St. Clair were also captured and released at the Thames River mouth. However, some of these yearlings were transferred and released a few miles further north. In 1956 and 1957, spawning-run walleyes were caught, tagged, and released in the Thames River, approximately 6 miles from its mouth.

Since the fish tagged in the Thames River were in a ripe or near-ripe sexual condition, the sex of most was recorded. Of 825 so examined in 1956, males made up 66%. Males also outnumbered females in 1957, when they constituted 73% of the 1879 examined.

The fish tagged in the Detroit River in 1958 were caught and released in the lower river within 5 miles of Lake Erie. Those released in Lake Erie during 1959 were all young-of-the-year that were caught on the west side of Point Pelee. Other walleyes released in Lake Erie during the years 1954 to 1957 were caught, tagged, and released near the ports mentioned in Table 1, except for Port Burwell, 1957. Here, the fish were caught by gillnets 20 miles southeast from this port, and approximately in midlake.

The ages of most tagged fish as shown by scale annuli were determined not only to provide knowledge of the population age structure, but also to see whether age biased recoveries. In general, the majority of walleyes tagged were age II or older (Table 1). Like those for the releases made upstream of the Detroit River (Table 2), the age structures of the fish recovered were generally similar to those of the fish released even though some were recovered several years later. This was not true for those fish tagged in Lake St. Clair during 1955; ages I and II made up almost 10% of those released, but provided no recoveries. On the other hand, fish older than age V made up 4% of the releases, yet contributed 18% of the recoveries in later years. It is not known why these young fish failed to provide recoveries, but a practise, at this time, of selecting mainly sublegal (less than 35.6 cm total length) fish for tagging may be a factor.

In the presentation of the results only general recovery areas are given in the tables and figures. This is faithful to the data, since recoveries tended to be scattered throughout each general area, with a partial exception occurring in Lake St. Clair. Here there was a tendency

for recoveries to be concentrated near the Thames River mouth. Seasonal and annual distributions were considered and particular attention was given to the dispersal of immature fish, those less than age III. The distribution of recoveries was not considered in relation to areal and temporal variations in the effort by sports and commercial fishermen. In general, the fishing effort by one or both means is intensive almost year-round throughout the study area.

Migrations from the Thames River

The migrations of walleyes that spawned in the Thames River were determined from a total of 422 recoveries from releases in April 1956 and March 1957 (Table 1). The seasonal distributions of recoveries were similar from the two releases (Table 3). For example, in the spring of 1956 and 1957 there were more first year recoveries from Lake St. Clair than from other areas. During the summer and fall, however, fewer fish were recovered in Lake St. Clair than in winter and spring, and more were taken from either the St. Clair River or southern Lake Huron. A similar pattern in the seasonal distributions of recoveries was observed in subsequent years.

The distribution of all recoveries indicated that the postspawning migration of walleyes from the Thames River was along a definite pathway extending northward from Lake St. Clair (Fig. 2). The largest single group of recoveries (32%) was taken from Lake St. Clair by a commercial fishery operating near the mouth of the Thames River. However, 45% of the total returns were taken upstream from Lake St. Clair, mainly in the St. Clair River and southern Lake Huron, a few being taken from Saginaw Bay. In contrast, only a small percentage of the returns (4.2%) came from downstream in the Detroit River and Lake Erie.

The postspawning migration of walleyes from the Thames River northward was rapid (Fig. 3). Walleyes soon left the river after being released (from late March to mid-April). In the last half of April more recoveries came from Lake St. Clair (34) than from the Thames River (15). Many were still being recovered from Lake St. Clair in the first half of May (34), but after mid-May few recoveries were made, suggesting that the walleyes soon left this lake. Commercial fishing in the lake continues almost until the end of May, but angling starts before this and is intensive throughout the summer. Therefore, had the walleyes lingered in Lake St. Clair, there would have been more recoveries here during the summer.

Many of the walleyes recovered from southern Lake Huron (17) were taken within 6 weeks after tagging (Fig. 3). The majority of recoveries from the St. Clair River were not made until June, which suggests that, initially, the walleyes did not remain in the river but moved directly into Lake Huron. However, more walleyes may have remained in the St. Clair River during May than suggested by the tag returns. The few May recoveries from the

TABLE 3. Thames River tagging. Numbers recovered in various areas according to season and year after release as spawning adults in 1956 and 1957.

Recovery area	1st year				2nd year				Later years			
	1	2	3	4	1	2	3	4	1	2	3	4
Tagged in 1956 (896 fish)												
Saginaw Bay	–	–	1	–	–	–	–	–	–	–	1	–
L. Huron	–	4	2	3	–	10	1	–	–	1	1	–
St. Clair R.	–	10	17	1	–	3	2	–	–	–	4	–
L. St. Clair	–	20	–	–	4	7	–	–	1	–	–	1
Thames R.	–	8	–	1	2	2	–	–	–	2	–	–
Detroit R.	–	–	4	–	–	–	–	–	–	–	–	–
L. Erie	–	–	–	–	–	–	–	–	–	–	–	–
Tagged in 1957 (1897 fish)												
Saginaw Bay	–	3	1	–	–	–	–	2	–	2	–	–
L. Huron	–	26	15	13	–	6	1	–	–	–	3	–
St. Clair R.	–	20	17	2	–	8	5	–	–	–	4	–
L. St. Clair	5	63	–	3	3	17	–	1	–	7	2	1
Thames R.	3	25	2	1	–	18	1	–	3	11	–	1
Detroit R.	–	3	3	–	–	2	1	–	–	–	2	–
L. Erie	–	–	–	–	–	2	–	–	–	1	–	–

river could be related to differences in fishing effort. All recoveries from the St. Clair River were by angling, whereas those from southern Lake Huron were by angling and commercial fishing. It is probable that angling intensity in the St. Clair River during May was not as high as the combined effort of sports and commercial fishing in Lake Huron.

FIG. 2. General movements of spawning adults released (2793) in the Thames River during April 1956 and March 1957. The recoveries (422) from each area are indicated as a percentage by the width of the arrow. The recoveries (18.9%) from the Thames River are not shown.

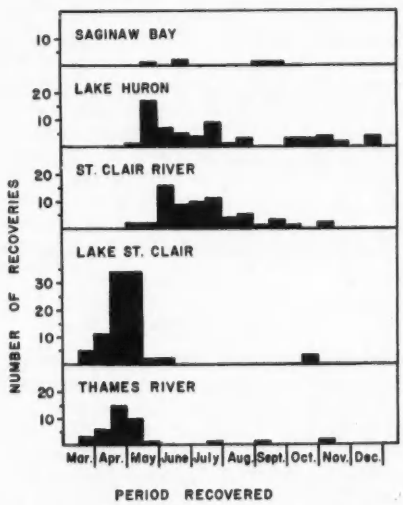

FIG. 3. First-year recoveries of adults released in the Thames River in April 1956 and March 1957. Numbers recovered are for semimonthly periods.

Recoveries from the St. Clair River and southern Lake Huron extended from May to December (Fig. 3). This long period of recoveries suggests that many walleyes remained in these areas through the summer. There is, however, an indication that some may have moved into Lake Huron after September. In October, November, and December, there were more returns from Lake Huron (16) than from the St. Clair River (3). The reduced number of recaptures from the St. Clair River may have been the result of reduced angling in these months. It is not known whether the walleyes overwintered in Lake Huron or in the St. Clair River since there were no recoveries made in January or February at any time. Those few that were caught in March were taken near the Thames River tagging site (Table 3).

Some individuals moved northward much faster than most others. One fish was recaptured in Saginaw Bay, a distance of approximately 175 miles from the tagging site in the Thames River, within 31 days. Two other fish were recaptured in the same area 60 days after release.

Tagging in Lake St. Clair in 1955, although resulting in only 8% recovery of the 458 walleyes, provided observed movements that were similar to those of the spawners tagged in the Thames River. Some (9) returns came from Lake St. Clair, but most (20) were from upstream: 13 in the St. Clair River, 5 in southern Lake Huron, and 2 in Saginaw Bay. Eight fish were recovered from downstream areas, four in each of the Detroit River and Lake Erie. Only one fish was recovered in the Thames River. It was recaptured during the 1956 spawning season, 6 months after release.

Age may have been a factor in the greater percentage (21%) of recoveries downstream from Lake St. Clair from the 1955 releases in Lake St. Clair than from the 1956–57 releases in the Thames River (4%). Most (66%) of the fish tagged in Lake St. Clair in 1955 were age III or younger, whereas those tagged in the Thames River were mainly older than age III (Table 2). In addition, downstream recoveries from those released in 1955 tended to be younger than the fish recaptured upstream from Lake St. Clair. The numbers of fish in the two age-groups that were recovered in the lake and upstream and downstream from it were:

Ages:	III and IV	V and older
Upstream	6	14
In Lake St. Clair	5	4
Downstream	6	2

Migrations from Southern Lake Huron

Walleyes tagged in Lake Huron were mainly recovered there, although almost as many were retaken in the St. Clair River (Table 4). This suggested a strong tendency to move downriver, further supported by the many recoveries from Lake St. Clair to Lake Erie. The four recaptured in the Thames River, during spawning, and the 27 further down the system suggest that two or more spawning stocks frequent southern Lake Huron during the summer and fall. Carr (1962) also reported a walleye that moved from Saginaw Bay to western Lake Erie.

Walleyes caught and released in the lower Detroit River were intended to supply information that would supplement that from the Lake Huron operation being carried out concurrently. Unfortunately, only a small number of fish were caught and released. Most recoveries were made in Lake Erie a short time later.

TABLE 4. Lake Huron tagging. Numbers recovered annually from various areas after release, mainly as age II fish, in 1958 and of totals recovered after release, mainly as age III fish, in 1955.

Recovery area	1958 release				1955 release
	1959	1960	1961–	Total	Total
L. Huron	43	11	1	55	19
St. Clair R.	36	5	2	43	5
L. St. Clair	1	3	2	6	–
Thames R.	3	–	1	4	–
Detroit R.	15	2	–	17	3
L. Erie	9	1	–	10	1
Totals	107	22	6	135	28

Two comments are pertinent at this point. First, there was no active fishery in the Thames River and the four recoveries there were fortuitous. This might suggest that more recoveries would have been made in this river if an intensive fishery had been operating there. Secondly, age may have been an important factor influencing the recovery trend of the 1958 release. Downriver recoveries (Table 4) in later years were predominantly mature fish. Most were age II in 1958 (Table 2).

Migrations of Juveniles from Lake Erie and Lake St. Clair

Fish age II and younger are here termed "juveniles." Preliminary appraisal of early results suggested that an attempt should be made to tag juveniles. Consequently the abundant year-class of 1959 provided 583 young-of-the-year from Lake Erie, and 712 (44 in February) yearlings from Lake St. Clair. The Lake Erie releases resulted in 115 recoveries (Table 5) during the next 4 years. During this period there was a temporal trend in the primary recovery area. Immediate recoveries suggested that the fish were dispersing rapidly. The following year (1960) this was confirmed when yearlings were recovered from all parts of the western basin, and 23% had travelled beyond this basin. This latter group consisted of almost equal numbers that had moved either along the U.S. shore of the central basin or into the Detroit River and beyond to Lake Huron (Fig. 4, top). The distribution of these fish in western Lake Erie seems to reflect the water current pattern of Olson as shown by Hutchinson (1957). At age II (1961), 46% of the recoveries were from beyond the western basin. Most of those were from Lake St. Clair, but some came from the Detroit and St. Clair rivers and from Lake Huron. One, significantly,

TABLE 5. Lake Erie tagging 1959. Percentage annual recoveries in various areas from 583 young-of-the-year walleyes released at west Point Pelee, and (in parentheses) from 3998 yearlings released by Wolfert (1963) in 1960 at various locations in the U.S. waters of this lake. Central Lake Erie comprises Wolfert's areas A and B and east of Lorain, Ohio.

	1959	1960	1961	1962	Total
Central L. Erie	3.6	12.4(24.2)	4.2(14.0)	–(15.8)	7.8(20.8)
Western L. Erie	96.4	76.7(71.5)	54.2(76.0)	–(47.5)	72.1(68.3)
Detroit R.	–	8.9(3.0)	8.3(6.0)	14.6(10.5)	7.0(4.2)
L. St. Clair	–	–(0.3)	25.0(4.6)	43.0(10.5)	7.8(2.0)
Thames R.	–	–(–)	–(4.6)	14.6(10.5)	0.9(1.8)
St. Clair R.	–	–(0.6)	4.2(6.0)	–(–)	0.9(2.2)
L. Huron	–	1.7(0.3)	4.2(0.7)	28.6(5.3)	3.5(0.6)
Total no. recovered	28	56(330)	24(150)	7(19)	115(499)

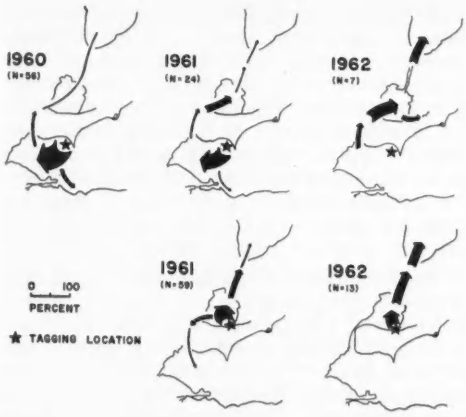

FIG. 4. Distributions of annual recoveries of 1959 year-class walleyes recovered at ages I (1960) to III (1962). The top panel shows the percentage recoveries in each area from 583 released as young-of-the-year at Point Pelee. The bottom panel shows the recoveries from 668 released as yearlings in Lake St. Clair near the mouth of the Thames River.

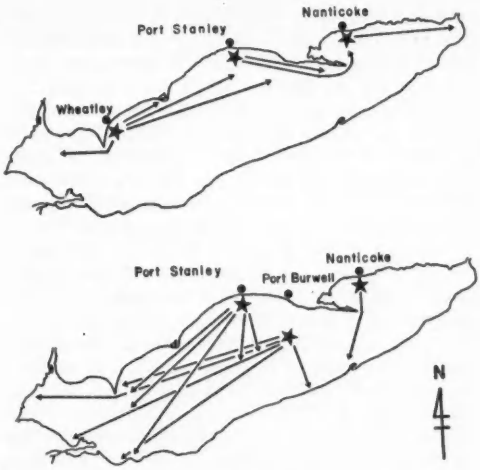

FIG. 5. Movements of individual walleyes in Lake Erie during selected periods, from releases at Wheatley (228), Port Stanley (232), Nanticoke (47) and midlake off Port Burwell (74). Stars indicate release points and arrow heads the recovery points. *Top* — summer movements prior to October 1 during the year of release; *bottom* — recoveries from March to May in subsequent years. *Note* — There were other recoveries at other times, but they contribute little to our understanding of migrations here.

was a male recovered from the Thames River during the 1962 spawning season. Two were from Lake Huron. The close similarity of these results with those of Wolfert (1963) is shown in Table 5.

Yearlings tagged when Lake St. Clair was ice-bound in February 1960, resulted in seven recoveries. One was recovered almost immediately from the tagging vicinity. The remainder were from the Detroit River and Lake Erie. A larger group of yearlings released in November 1960, provided 74 recoveries. These also showed a temporal trend in primary recovery area (Fig. 4, bottom). At age II they were recovered in about equal numbers downriver and upriver from Lake St. Clair, suggesting dispersal in both directions. At age III there were no recoveries from downriver; all recoveries were from upriver, some from as far as Lake Huron.

Migrations in Lake Erie

The results from the tagging in Lake Erie at four different locations from 1954 to 1957 were combined since they are similar. Almost all recoveries in the year of tagging were from east of the tagging site (Fig. 5, top). Some had travelled a considerable distance before being recovered. These fish were recovered prior to October 1 and, therefore, may be said to represent the summer movement. Subsequent recoveries are of limited value for discerning direct migrations, but those made in March, April, and May (Fig. 5, bottom), encompassing the spawning period, suggest the variety of general spawning locations utilized by these fish. Most were recovered in, or approaching, the western basin, but some had moved south across the lake towards the U.S. shore. These fish were mainly age II when tagged (Table 1). Their average age when recovered was IV.

Discussion

The impetus for this work stemmed from the need for information on the movements and mixing of walleye stocks, particularly those spawning in the Thames River. From our point of view, therefore, stocks were identified with their "home" spawning stream, or spawning location. Since we could only rely on two point captures (release and recovery points) this necessitated a multiplicity of release points to obtain the necessary information on movements throughout the study area. However, we were restricted in the locations and times when we could capture and tag these fish. The effort to obtain walleyes in sufficient numbers and in sufficient areas was to some degree happenstance. Inevitably, some areas were not covered, or not

covered ideally. The results reflect this "shotgun" approach, and our understanding is correspondingly fragmentary. However fragmentary these results may seem, they do represent a major advance in our understanding of the movements and mixing of these stocks of fish. The fact that releases and recoveries were spread over more than 10 years seems to be of less consequence than the geographic gaps, or the lack of more information on the movements of young-of-the-year walleye.

The movements of adult walleyes in the early spring and summer present the clearest results. These results (Fig. 6A) were not only clear, but were repeated during several years tagging. Adults from the Thames River almost invariably moved into and through the St. Clair River. It is obvious also that many of these fish remained in this river (Fig. 3). Most of those that travelled to Lake Huron appeared to remain in the southern portion of that lake, within 20 miles of the river, although a few continued on to Saginaw Bay. Adults in Lake Erie were not tagged at their spawning sites, but during early and midsummer. They were therefore intercepted while they were apparently migrating east along the north shore of this lake. The small numbers of fish involved in this part of the study (Table 1) do not appear to invalidate it since the results each year support the general conclusion. The small numbers tagged do not permit assessment of the rate at which these movements took place, or of whether all fish were continually moving east or whether some remained for prolonged periods in certain locations. These

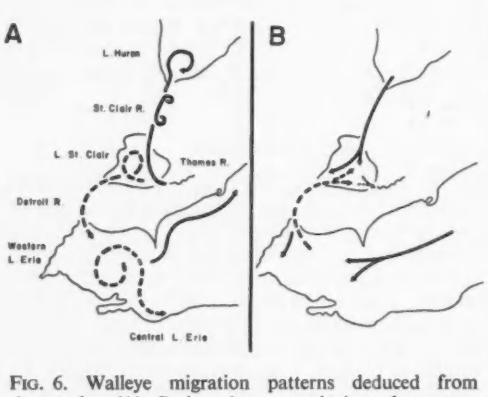

FIG. 6. Walleye migration patterns deduced from the study. (A) Spring (postspawning) and summer migrations of adults (solid lines) and immature (broken lines) fish. Whorls suggest milling areas. (B) Fall to early spring (prespawning) migrations. Broken lines here indicate maturing fish that have not spawned previously.

results were closely similar to those obtained by Ohio biologists who were also tagging walleyes during this period (R. Cummins personal communication). They released large numbers of walleyes in the Kelly's Island area of Lake Erie during the 1950 decade. Our Lake Erie tagging was completed before the catastrophic decline in walleyes in this lake (Baldwin and Saalfeld 1962, and Regier et al. 1969). It may be significant that these fish showed a positive northeast migration along the Canadian shore during the summer.

The movements of adults during the fall and early spring were, in general, the reverse of those shown in the spring and summer, suggesting the return of these fish to their spawning areas (home stream or home shoal). They do offer additional information on the stock mixing that must have occurred during the summer. For instance, walleyes moving from southern Lake Huron (Fig. 6B) showed three destinations: two general areas, western Lake Erie and southern Lake Huron, and one specific location, the Thames River. Western Lake Erie was also the apparent spawning destination of many of the adults tagged in Lake Erie. This is corroborated by Canadian commercial fishermen's observations, and the seasonal landings of the species in various statistical districts on the north shore (Regier et al. 1969). Manz (1964) found western Lake Erie a major spawning area. Early spring recoveries also suggested that the south shore of central Lake Erie was the destination of some fish. This, too, is corroborated by the seasonal landings by Ohio commercial fishermen.

The movement of juvenile fish is not nearly as clear as that of the adults, but it does appear to fit the general pattern that emerges. Young-of-the-year and yearling walleyes in Lake St. Clair did not behave in the same manner as adults. They showed a pronounced tendency to move down the Detroit River into Lake Erie. This portion of the migrations requires more detailed study. Age appeared to exert a strong influence on whether the fish went downriver or up. Fish of age I and older showed a progressively stronger tendency to migrate up through the system into the St. Clair River and Lake Huron. Undoubtedly some fish that initially moved down into Lake Erie, returned later, after milling in western Lake Erie. Some young-of-the-year walleyes tagged by us in the western basin of Lake Erie could therefore have been fish that originated in Lake St. Clair. The milling of young walleyes in Lake Erie appeared almost restricted to the western basin as shown by our results, and also those of Wolfert (1963). The slight amount of wandering into the central basin along the south shore of the central basin is only

notable in that it was so clearly divergent from the migrations of the adults. In this instance as well as in Lake St. Clair it seems clear that adults and juveniles dispersed in almost diametrically opposite directions during the late spring and summer (Fig. 6A). This suggestion is supported by Parsons' (1970) comparison of walleye ages from catches made at Sandusky, Ohio, and Wheatley, Ont.

The fall and early spring movements of juveniles are not well documented by our results, partly because too few were tagged, but also because at age II they were apparently behaving almost like adults. Wolfert (1963) found one male was mature at age III and was spawning in the Thames River, adding further indication that fish from Lake St. Clair may move into Lake Erie at an early age. Although our data suggest that the 1959 year-class in western Lake Erie probably originated in Lake St. Clair and the Thames River, Wolfert's results suggest that many of his fish originated from spawning grounds in western Lake Erie and remained there. They were being recovered there at a much older age, at a time when all our recoveries were being made upriver, in the Detroit River, St. Clair River, or Lake Huron.

These results do suggest that both southern Lake Huron and western Lake Erie are areas where walleye stocks are extensively mixed during the summer. Southern Lake Huron is mainly an area where adult stocks from that area, the Thames River, and western Lake Erie mix. Western Lake Erie, and to some extent the southern shore of central Lake Erie, is the area where juvenile stocks mix. In western Lake Erie, mixing is an active process with juvenile fish appearing to move rapidly throughout the basin, but seldom leaving it. The north shore of Lake Erie during the study (1950 decade) appeared to be a ranging area with stocks of adult fish mixing but continuing to move east rather than remaining in any area. The ranging is a suggested but plausible explanation of the results, since the data are sparse. Doan (1942) tagged walleyes in Lake Erie, and although his data are also sparse and he does not specify ages, his results appear to corroborate ours and Wolfert's (1963).

A number of authors, Gerking (1959), Forney (1963), and Magnin and Beaulieu (1968), have emphasized the importance of the summer "home" or feeding area of this species in the populations that they have studied. It is true that the summer sojourn is the longest period that the fish spend in any given location, but it has little significance according to our understanding of stock well-being. The summer aggregations represent, or may represent, conglomerates of spawning stocks. It is also apparent that we must know something of the migratory habits of the juveniles quite separately from those of the adults.

It appears of little consequence to emphasize one "home" area over another; rather, it would seem most important to adopt a scheme similar to that of Nikolsky (1963). He speaks of the "feeding," "overwintering," and "spawning" migrations. Perhaps it would be more correct to consider these migrations to a feeding, overwintering, or spawning area. By defining the duration of each, it should be possible to better understand and manage such stocks of fish.

In the present instance adults appear to frequent their feeding areas from May to November. The overwintering area is not clearly defined by our results but, if it occurs, it would be December to March. The period in the spawning area is short, March and part of April, but is perhaps considerably shorter for females than males.

Acknowledgments

The stimulation and support of the late Dr W. J. K. Harkness and of Dr J. K. Reynolds, whose particular interest in the Thames River stock prompted this study, are herewith gratefully acknowledged. Mr J. D. Roseborough and members of the Lake Erie District staff assisted in all tagging operations in the Thames River and in the Lake Huron work in 1958. Messrs L. Drummond, D. Frederisy, J. Kippax, and R. Lowe assisted in all phases of the tagging operations. Mr Kippax also assisted by maintaining the tag return records. Special thanks are due the late Mr K. Kodaira for devising and drafting the figures. We are indebted also to the many sport and commercial fishermen who reported recoveries of tagged fish. Redaction of the manuscript by Drs V. C. Applegate, J. L. Forney, and H. A. Regier, and Messrs K. H. Loftus and R. A. Ryder enhanced this presentation.

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Locomotory Mechanisms in Caligidae (Crustacea: Copepoda)

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KABATA, Z., AND G. C. HEWITT. 1971. Locomotory mechanisms in Caligidae (Crustacea: Copepoda). J. Fish. Res. Bd. Canada 28: 1143–1151.

Structure of locomotory appendages of Caligidae (Copepoda: Caligoida) is briefly reviewed from the point of view of functional morphology. Locomotory mechanisms are studied on examples of *Lepeophtheirus salmonis* and *Caligus clemensi*. Rapid propulsion is attributed to antagonistic movements of the first two pairs of legs, the second pair being a more important contributor. Beating towards each other, the legs produce a jet of water, which propels the parasite. In a subsequent recovery stroke, the second leg, made watertight by a dorsal membrane, gives further impetus to the jet, pushing it out through the sinus opening of the cephalothorax. Water is replenished under the cephalothorax by intake via space under the frontal plates. Settling movements help to improve the grip of the parasite on the host's surface and involve rapid side-to-side motion, involving the second maxillae. Steering is carried out by lateral and vertical movements of the posterior part of the body. Functions of other appendages are discussed.

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PARASITIC copepods belonging to the family Caligidae are among the most common crustacean parasites. They are numerous in species and frequently abound on the surfaces of their hosts. Some have been known for nearly 200 years. When present on fish in large numbers, they can have a grossly damaging effect on the infested areas of skin. In some well-documented instances they have been implicated in mass mortality of fish (e.g., White 1940).

It is, therefore, surprising that next to nothing is known about the biology of these parasites. Although they are common enough to be fairly easily obtained as experimental subjects, they have rarely been studied alive. Most of the work done on live caligids has been concerned with developmental stages. Working with adults, Krishnaswamy (1960) studied the toleration of anaerobic conditions by *Caligus diaphanus*, and Berger (1970) investigated the effects of salinity changes on *Lepeophtheirus salmonis*. Comments on the physiology of *Caligus savala* were made by its discoverer, Gnanamuthu (1948), who also touched upon some aspects of the parasite's behaviour. Schuurmans Stekhoven (1934) studied the sensory physiology of *Lepeophtheirus pectoralis*. His paper described also several distinctive patterns of the parasite's movements, without considering the mechanism of these movements.

Caligids can swim freely through the water. They can also move quite rapidly over the surfaces of their hosts. Although the structure of their appendages provides some clues as to the roles they play in causing movements, they have never been studied in action.

There are at least three reasons for wishing to know more about the parts played by individual appendages in the locomotion of the caligids. First, the way in which appendages are used may be directly responsible for the effect they produce on the attachment surface and, therefore, be of interest to those concerned with fish diseases. Second, the appendages of Caligidae are commonly used in taxonomic studies. The importance of individual appendages for taxonomy will depend in part on their susceptibility to selection pressures, which in turn is a function of the way in which the appendages are used. Third, the subject has its own intrinsic interest.

[1]This work was conducted when the author was at the Nanaimo Biological Station as an N.R.C. Postdoctoral Research Fellow.

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It is the object of this paper to describe the results of a set of observations carried out by the authors on live caligids belonging to two different species, special attention being paid to the locomotory mechanisms operating during free movement as well as while in contact with the surface of the host or other substrate. Since all members of the family Caligidae have appendages of close structural similarity, it is fair to assume that the results of these observations can be extended to include other caligids.

Methods

Observations were made mainly on *Lepeophtheirus salmonis*. They were supplemented by some observations on *Caligus clemensi*. The copepods were transferred as soon as possible after capture to sea water, changed twice a day, and maintained at 5 C. *Caligus clemensi* proved difficult to work with due to its sensitivity to temperature changes and inability to perform or even to survive for long at room temperature. *Lepeophtheirus salmonis*, on the other hand, was an excellent subject. When exhausted by repeated stimulation, it could be returned to low temperature storage where it recovered sufficiently to be used again the following day. It survived under those conditions for up to 10 days.

The observations were made using the following approaches.

(1) *Caligus clemensi* (a common parasite of herring) was observed while attached to the threespine stickleback, *Gasterosteus aculeatus*, and to the shiner perch, *Cymatogaster aggregata*, in a seawater aquarium. *Caligus clemensi* attached to sticklebacks were also studied under a dissecting microscope in a large Petri dish.

(2) *Caligus clemensi* and *L. salmonis* were observed in a horizontal staining dish. The parasite was caused to attach to a glass slide. With the slide turned over and resting at an angle in the dish, the ventral surface of the parasite was exposed to the observer.

(3) *Lepeophtheirus salmonis* was studied with various appendages or combinations of appendages amputated or immobilized. Immobilization was effected with the aid of Loctite Quick Adhesive 404 (Loctite Corp., Newington, Connecticut, USA). The experimental specimen was removed from water and placed on a slide under a dissection microscope. A small drop of glue was placed on the base of the limb to be immobilized. The entire operation took only a few seconds and the copepod was then immediately returned to water and placed at low temperature for about 30 min. After this period the parasite recovered and resumed active movements when stimulated.

(4) Both *C. clemensi* and *L. salmonis* were studied from the ventral side with the aid of a Wild inverted microscope. Attempts were made to record their movements with the aid of a cinecamera.

(5) Specimens of both species were placed, ventral side up, in a small Petri dish containing sea water and their movements observed with the aid of a dissecting microscope and a stroboscope.

(6) Specimens of *L. salmonis* were observed in a dish, the bottom of which was lined with various grades of sandpaper.

(7) A small quantity of India ink was introduced into a Petri dish just in front of specimens of *L. salmonis* and the movement of the ink was watched, when the parasite was stimulated to move.

(8) The swimming motions of *L. salmonis* were studied in a narrow glass tube filled with sea water and held in vertical position.

Appendages and Structures Associated with Locomotion

A detailed description of the morphology of the caligid appendages was given by Parker et al. (1968), who described *Caligus curtus*, the type species of its genus. This paper, therefore, gives only a brief account of the general structural plan of the appendages and other structures associated with locomotion, describing them with the aid of diagrams based on Parker's work. To assist the reader's memory about the general structure of the caligids, one of Parker's figures is reproduced in this paper (Fig. 1).

The second maxilla (Fig. 2) is long and slender. It can be likened to a human arm, its distal and proximal parts flexing upon each other in an elbowlike joint. It is tipped with two long, flexible processes and is normally situated in front of the prominent maxillipeds, positioned across the body and flexed as in Fig. 2.

The first leg (Fig. 3) is long and slender, uniramous, though it is equipped with a very small vestigial and functionless endopod. The exopod is large and is the main working ramus. The leg is usually observed in the position in which its margin, shown as lower in Fig. 3, points posteriorly. It is capable of swinging through an arc that would bring this margin pointing obliquely forward. The two limbs of this pair are connected by an interpodal bar and move always together. The tip of the exopod is armed with four modified setae (x), the structure of which varies from species to species. Its posterior margin bears three pinnate setae (y), present in most caligids, though slightly modified and reduced in some and absent in a few.

The second leg (Fig. 4) retains its relatively primitive biramous condition. Like the first, it is linked by an interpodal bar with its opposite number, and the pair work as a unit. Several structural features of this limb should be noted in connection with the function it performs. First, when the limb is at right angles to the ventral surface of the body, its exopod is slightly posterior to its endopod, their bases partly overlapping. Secondly, the endopod is capable of swinging inwards so as to become nearly parallel with the lower margin of the sympod. When in this position, it turns the lateral

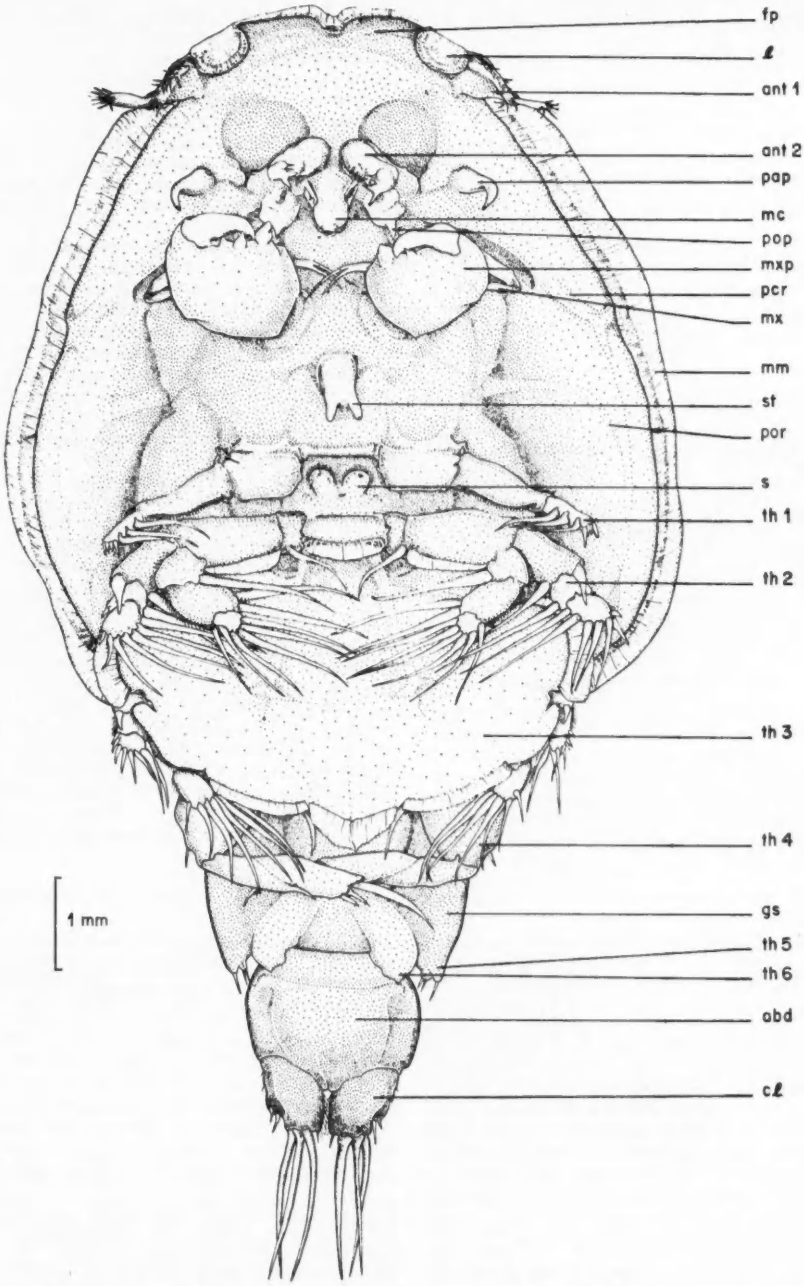


FIG. 1. *Caligus curtus*, male, ventral (from Parker et al. 1968). *abd*, abdomen; *ant* 1, first antenna; *ant* 2, second antenna; *cl*, ramus of caudal furca; *fp*, frontal plate; *gs*, genital segment; *l*, lunule; *mc*, mouth tube; *mm*, marginal membrane; *mx*, second maxilla; *mxp*, maxilliped; *pap*, postantennary process; *pcr*, posterior cephalic ridge; *pop*, process of first maxilla; *por*, posterior ridge; *s*, swelling; *sf*, sternal furca; *th* 1–*th* 6, first to sixth thoracic legs.

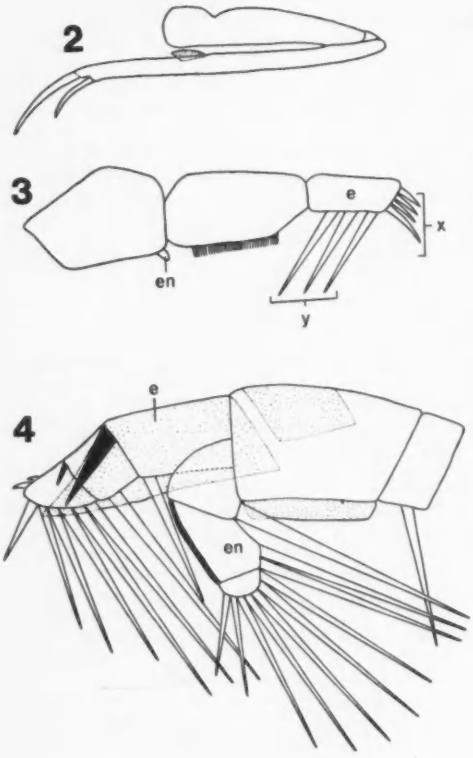


FIG. 2. Second maxilla, diagrammatic.
FIG. 3. First thoracic leg, diagrammatic.
FIG. 4. Second thoracic leg, diagrammatic. *e*, exopod; *en*, endopod; *x*, terminal setae; *y*, posterior setae.

margin of its second segment (marked by a thick black line in Fig. 4) towards the substrate. This margin is variously armed in different caligid species. In some of them it possesses merely a fringe of fine, hairlike setules; in others the setules are coarse and thick; in yet others the margin is armed with a row or several rows of spines, sometimes quite formidable (e.g., *Caligus productus*). Thirdly, a large part of the posterior (or dorsal) surface of the limb is covered by two membranes (stippled in Fig. 4) attached to the upper margin of the limb, a smaller one to the sympod and a larger one to the proximal segment of the exopod. The latter stretches almost to the tip of the exopod. Fourthly, the proximal and middle segments of the exopod carry distally a large spine each, particularly prominent on the proximal segment. When the endopod is retracted and the leg situated obliquely backwards, these spines find themselves in contact with the substrate. The spines are articulated and capable of some, though limited, movements. Fifthly, the pinnules of the long, pinnate setae are placed closely together and can be made to overlap by the appropriate positioning of the setae, so as to form a fairly solid wall, enlarging the surface of the leg. When the endopod is retracted, the tips of its setae overlap those of the opposite leg, as can be seen in Fig. 1. A similar inward flexion of setae is possible for the exopod. When so retracted, the rami present to the substrate the lateral sides of their most lateral and shortest setae. It is these setae that are often modified along the lateral margins and carry on them instead of the usual row of pinnules a strip of membrane or coarser denticulation.

The third leg (Fig. 5) is profoundly modified by complete fusion of the two members of the pair. The sympods, with the space between them filled by a posterior expansion of the interpodal bar (obliquely striped in Fig. 5) form a transverse barrier closing off the posterior end of the cephalothorax (Fig. 1). The rami of the third leg are vestigial and can be retracted flush with the margins of the legs. The entire margin, with the exception of the space between the rami, is fringed by a strip of membrane. The remaining space is filled

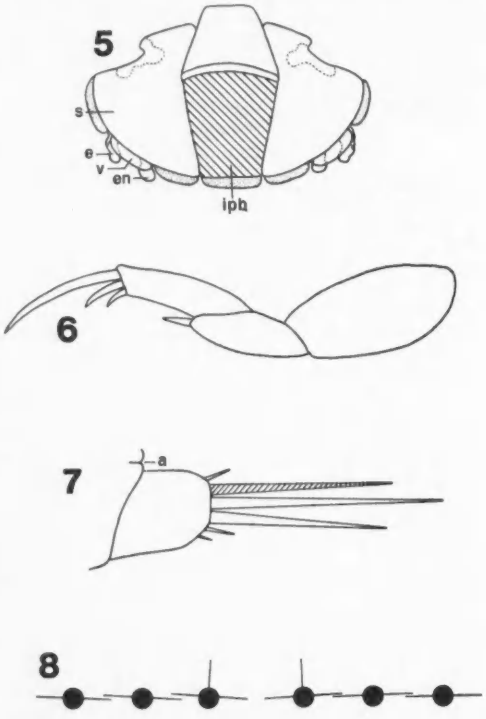


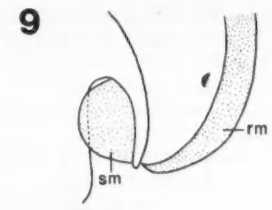
FIG. 5. Third thoracic legs, fused, diagrammatic.
FIG. 6. Fourth thoracic leg, diagrammatic.
FIG. 7. Caudal ramus, diagrammatic.
FIG. 8. Transverse section through setae of caudal furca, diagrammatic. *a*, anus; *e*, exopod; *en* endopod; *ipb*, interpodal bar; *s*, sympod; *v*, velum.

by a sclerite described as a velum by Parker et al. (1968) and extending on both sides between the rami. The third leg usually points posteriorly with its free margin and is capable of only limited movements.

The fourth leg (Fig. 6) is uniramous and roughly resembles the structural plan of the second maxilla. It is also capable of flexing upon itself due to possession of an elbowlike joint between the proximal segment and the rest of the limb. Its tip is armed with setae varying in number from species to species, though never more than three. There are either one or two setae of similar type on the lower margin of the distal part of the leg. The leg differs from the preceding pair by being outside the cephalothorax and separated from the preceding appendages by the barrier of the fused third legs.

The caudal furca consists of two rami (Fig. 7). Each ramus is a quadrangular sclerite, equipped with six setae, three short and three long and pinnate, usually much more prominent in the males. The pinnules can overlap, as in the setae of the legs, to form a fairly broad surface. The inner seta of each ramus (obliquely striped in Fig. 7) has a third row of pinnules on its dorsal side. (In some specimens this is displaced from dorsal by rotation of the seta.) The six long setae of the caudal furca appear in cross-section as shown diagrammatically in Fig. 8.

The cephalothorax of the caligid copepods is modified to act as an efficient suction cup. Its anterior margin is prolonged into two flat frontal plates (Fig. 1) and the lateral margins are rimmed with a broad strip of transparent membrane, richly equipped along its base with fine setules and in some species modified to extend for some distance below the margin of the cephalothorax. In the posterior margin are situated two openings, known as posterior sinuses (Fig. 9). They are provided with flaps of membrane (*sm*) that can be deflected dorsally by a stream of water issuing from beneath the cephalothorax but close the sinus opening under pressure exerted by water external to it. In effect, the sinus is a one-way valve.



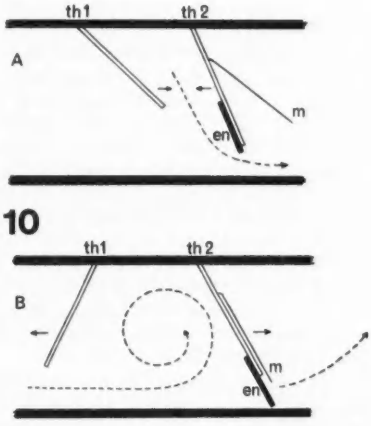


FIG. 9. Posterolateral corner of dorsal shield, showing membranes (diagrammatic).

FIG. 10. Diagram illustrating movements of first and second pair of thoracic legs: (A) effective stroke, legs beating towards each other; (B) recovery stroke of first pair, second phase stroke of second pair (explanations in text); *en* endopod; *m* membrane of second leg; *rm* rim membrane; *sm* sinus membrane; *th* 1, *th* 2 first and second leg.

Mechanism of Rapid Propulsion

Little observation is required to establish that the appendages responsible for the rapid movement of the caligid copepods are the first and second pairs of legs. Because of their extreme rapidity, the manner in which these appendages cause the copepod to move, is, however, more difficult to determine. Free-swimming in an aquarium, *C. clemensi* was timed at 100 beats per minute. To learn about the movements of the first and second pairs of legs, it was necessary to observe the lower surface of *L. salmonis* crawling over an inverted slide, under a dissection microscope. Even here, the rapidity of their movements made the legs difficult to observe. The stroboscope proved a substantial help in this observation. As might have been expected, the two pairs of legs do not beat simultaneously. As is well known, the free-living copepods move their natatory appendages in a metachronal rhythm. The sequence of movements has been changed in the caligids, so that the two pairs of legs beat against each other. The way in which this motion is translated into forward movement is shown in Fig. 10. When motionless, the first pair of legs points slightly forwards and the second pair obliquely backwards. In the first phase of their beat, constituting the effective stroke (Fig. 10A), the two pairs beat towards each other. At the end of the stroke they both point posteriorly and their approximation results in a jet of water being squeezed from the space between them and squirting backwards and downwards. In a free-swimming copepod this jet provides both the forward and the lifting component. In the copepod crawling over the surface of its host the

jet passes under the free margin of the second leg and into the space under the posterior part of the cephalothorax. The endopod of the second leg is pulled up to the highest position possible. Some of the water might pass between the setae and under the membranes, which at that time are being trailed behind the leg and are at an angle with it.

With the effective stroke completed, the legs beat away from each other in the recovery stroke. At least this might be called a recovery stroke for the first legs. As the two pairs move away from each other, the space between them becomes larger and water is drawn into it under the free margin of the first leg (Fig. 10B). The endopod of the second leg is lowered so that it touches, or almost touches, the substrate. The membranes are pressed tightly against the leg and make it watertight. Water displaced during the previous stroke as a posteriorly directed jet is now pushed further in a posterodorsal direction (Fig. 10B). (It should also be kept in mind that in the attached parasite the recovery stroke can also be accompanied by scraping of the substrate by the lateral margin of the second segment of the endopod of the second leg. This might act as part of the propulsive mechanism, pushing the animal forward. It might also irritate the fish's skin and stimulate the production of mucus.)

The backward propulsion of the water by the legs can, therefore, be described as a two-phase operation, the first phase of which is carried out by both pairs of legs, whereas the second phase is the responsibility of the second legs alone. In the free-swimming copepod one can presume that the first phase has the main locomotory effect, since the jet is not confined in a close space where it can be acted upon by the backward sweep of the second leg. In the crawling animals, however, the second phase is much more effective.

The movement of the jet pushed backwards by the second phase of the second leg's movement increases water pressure under the posterior part of the cephalothorax, between the second and third legs. This causes the one-way valves of the posterior sinuses to open and jets of water to issue from them backwards and upwards, contributing to the forward movement of the copepod and at the same time helping to press it against the surface of the host.

The water ejected backwards by the successive strokes of the second and first legs is replaced under the cephalothorax in a crawling copepod by water taken in under the frontal plates. Specimens attached to a flat glass surface and presented with a drop of India ink were observed to take in ink with the water at the beginning of active movement. Water was drawn in by the animal slightly raising its frontal plate and flexing dorsally the anterior part of its cephalothorax at the transverse hinge between the thoracic and cephalic areas of the cephalothorax (see Parker et al. 1968). The ink was then seen entering the space between the first and second legs and being ejected in a jet by the first phase of their beat.

Although both the first and second pairs of legs are active in producing this motion, it appears that they are not equal partners. This became apparent when legs were immobilized in turn. When the first pair was rendered inactive, the animal could still swim and crawl along a glass surface, although at a reduced speed. This is probably due to the fact that even without the assistance from the first pair the second pair is capable of pushing water backwards by the second phase of its stroke. When the second pair was immobilized, however, the animal was no longer capable of progressing in the normal manner, since the beating of the first legs alone could not produce effective movement of the water.

"Settling" Movements

When attached to a stationary or slow-swimming fish, the caligids perform, immediately after attachment and then at intervals, a set of movements referred to by the authors as "settling" movements. Similar, though less frequent and less vigorous, movements are also carried out by copepods attached to the vertical walls of glass containers. Immediately before the movements begin, the valvelike membranes of the posterior sinuses open, indicating increase of water pressure under the posterior part of the cephalothorax and, consequently, the movements of the first and second legs. This is followed by several slight movements rotating alternately to the right and left of the longitudinal axis of the body. A slight forward shift of position takes place. The general impression conveyed by these movements is that the animal tries to strengthen its grip on the host by bringing the margins of its cephalothorax into close and contiguous contact with the substrate, i.e., by sealing the suction cup and then increasing the suction force by expelling water from the cup.

Since the settling movements involve repeated and rapid changes of direction in which the copepod is facing, they cannot be accomplished by beating of the first and second legs alone. Since these appendages can move only together (i.e., both right and left legs simultaneously), they cannot be effective in direction changes, though it is partly due to them that the forward displacement takes place. Observations of the ventral surface of *L.*

salmonis have shown that these deflecting movements are effected by the second maxillae (Fig. 2). These long and slender appendages twitch rapidly in the course of settling movements, their motions being comparable in character, though not in speed, to the movements of the flexed arms of a crawling man. Moving alternately, they push forward and sideways first one side of the copepod, then the other.

That the second maxillae are capable of moving a caligid forward, however slowly and laboriously, could be seen in specimens with both first and second legs immobilized. These specimens crawled forward by extending their maxillae alternately, until they protruded from under the frontal plates. The body was then dragged forward by the flexion of the maxilla. This crawling also involved slight deflection to the right and left from the longitudinal axis, corresponding to the movements of the left and right maxilla respectively.

As already stated, the settling movements strengthen the contact between the caligid and its host. Such movements were rarely observed in caligids attached to vigorously moving fish. When attached to the fish, caligid copepods always face the direction in which the fish is moving, i.e., they are directed with their heads against the current of water. The cephalothorax of a caligid copepod is, under such circumstances, closely attached to the skin of the fish, whereas its genital segment and abdomen (and the egg-sacs of the female) lift off the surface of the fish and trail in the slipstream. It can be supposed the stream serves to press down the cephalothorax. At the same time it counteracts the force of gravity in maintaining the body in a horizontal position. When the fish is moving sluggishly or is stationary, the weight of the posterior half of the body will tend to make it droop towards the bottom and will make the efficient suction of the cephalothorax more difficult. Hence the settling movements, repeatedly improving the suction and perhaps also correcting undesirable changes in the position of the body caused by the drooping of the genital segment. When attached to the vertical glass walls of aquaria, caligids are usually in vertical positions. Even in the complete absence of the slipstream, these parasites are subjected to directionally different effects of the force of gravity, not involving lateral displacement of the posterior part of the body and, presumably, not having the same detrimental action on the holding properties of the cephalothorax. The authors observed that these parasites perform settling movements far less frequently.

The fact that the suction of the cephalothorax depends on the maintenance of intimate contact between its margins and the substrate has been demonstrated by making specimens of *L. salmonis* settle on strips of coarse sandpaper. They were completely unable to become attached to it and, when prodded from behind, they simply floated off the paper. When attached to the skin of teleost fishes, even the rough-skinned ones, the caligids are aided by the presence of mucus stimulated by their attachment. (Pandaridae, copepods structurally fairly close to the caligids and living on the skin of elasmobranch hosts, are faced with the necessity of attaching themselves to coarse surfaces on which mucus is sparse or nonexistent. Perhaps, in connection with the difficulty of maintaining a watertight seal under those conditions, these copepods have developed accessory organs of attachment, known as the "adhesive pads.")

The settling movements are obviously of great importance in the maintenance of the host–parasite system. They are also important for the establishment of new such systems. The caligids, as mentioned below, tend to be "restless" on the slow-swimming or stationary fishes. Many fishes remain stationary for quite long periods, as observed by Hobson (1965). It is during these periods that the caligids might be expected to change their hosts. One of the authors (G.C.H.) frequently took both male and female caligids in plankton nets at night, when diurnal fishes are not very mobile. Host changes are probably not uncommon during such periods.

Steering

Free-swimming caligids are capable of changing direction both in the horizontal and vertical plane. These changes are caused by alterations in the position of the posterior part of the body relative to the cephalothorax. The suggestion that emission of water jets from the posterior sinuses might be instrumental in effecting changes in direction of movement was rejected when specimens, with either one or both sinuses rendered inoperative by glue, were observed to swim and steer normally.

Ventral flexion of the posterior part of the body causes a caligid to dive, whereas its dorsiflexion produces upward swimming. Moving to the left, the posterior part produces leftward deflection and vice versa. The genital segment and abdomen, then, act jointly as a rudder. It appears that this action is augmented by the caudal furca, with its three long pinnate setae. It should be noted that the vertical rows of pinnules (Fig. 8) on the inner seta of each ramus can act as vanes assisting in horizontal steering.

Neither free-swimming nor attached caligids are capable of reversing their direction by moving

backwards. Adapted to maintain their hold against the current, they have developed devices protecting them against slipping backwards. Specimens on coarse sandpaper, incapable of attachment when prodded backward, remain stationary. It can be presumed that in such event all sharp-pointed structures (postantennary spines, the first maxillae, sternal furca, the lateral margins of the second endopods, the fourth legs) ram obliquely into the substrate in an effort to prevent slipping (Fig. 1). To retrace its course, a caligid must turn about.

Gnanamuthu (1948) suggested that caligids crawling over the surfaces of their hosts are incapable of changing their direction. To this inability he ascribed the fact that *Caligus*, left in an aquarium, sometimes climbs its vertical walls out of water and remains there until dead of dessication, apparently unable to return. This curious "suicidal" tendency is worth investigating, but it does not have to be the result of any deficiencies in the locomotory mechanisms. *Lepeophtheirus*, a parasite virtually identical with *Caligus* in structure, will not climb out of water under similar circumstances. The ability to "reverse" is not essential to avoid leaving the water, since the parasite is quite capable of turning around. This capability is not immediately affected by leaving water, as can be seen in caligids vigorously crawling (and changing direction) on the skin of a newly caught fish.

Comments on Functions of Other Appendages

The first antennae of the caligid copepods (Fig. 1) appear to be purely sensory and to play no part in propulsion. When the animal moves along a substrate, the distal segment of the first antenna can be seen to move repeatedly in the dorsoventral plane, rising from the substrate and touching it some distance further ahead. The armature of the apex of the first antenna consists of eight robust and moderately long or short setae, grouped roughly on the anterior half of the apex, and of five long flagelliform setae that occupy the posterior half. Two of the latter group arise out of a common base. The differences in the structure of these two groups of setae are greater or lesser, depending on the species, but are noticeable in all caligids. In some (e.g., *Caligus coryphaenae*) the posterior setae are plumose and very long. When crawling, the anterior group touches the substrate first and the posterior group tends to trail over the ground already covered. Some rotating movements of the distal segment around the longitudinal axis of the first antenna have also been observed.

The second antennae and the maxillipeds are strong, rapaciously prehensile appendages. Their exact functions were not determined in the course of these observations, since their normal functions could not be performed on glass surfaces. It is quite probable that the second antennae are used in particular as "grappling irons" when the copepod first settles on the fish. Both they and the maxillipeds can also be used in emergencies, when the suction of the cephalothorax has to be temporarily relinquished for any reason. The maxillipeds might also be used in procuring food. In the male, both the maxillipeds and the second antennae are used during mating.

The third pair of legs (Fig. 5) is not used in locomotion but plays an important part in the process of attachment. It has been observed to move its free margin slightly forward or backward. This movement was accompanied by the raising and lowering of the posterior part of the cephalothorax respectively. When settling, the parasite completes its attachment process by sliding forward the free margin of its third legs and enlarging the space under the cephalothorax. This presumably lowers the pressure under it and increases the force of suction.

The fourth pair of legs (Fig. 6) appears to be least used of all. With the exception of braking action, carried out when the parasite is forced backwards, they appear to be mainly inactive. When braking, the fourth legs fold transversely across the ventral surface of the genital segment and their lower margins are brought into contact with the substrate, presumably engaging their setae or, in some species (e.g., *Lepeophtheirus sturionis*), denticles with which the lower margin of the distal part is armed. With increase in backward pressure, the fourth legs are employed in alternate backward beats, clearly aimed at resisting slipping. On one occasion the left fourth leg was seen extending laterally and moving forwards over the surface of the cephalothorax in an apparently sweeping motion. Since this type of movement occurred only once during the course of observations and was not subsequently elicited by any of the stimuli applied, the authors make no suggestions as to its nature.

The function of the caudal furca in assisting changes of direction during swimming and crawling was described in the preceding section. The authors observed also, as did Schuurmans Stekhoven before them (1934), that *Lepeophtheirus*, sinking to the bottom of the vessel, is provoked to vigorous upward swimming when the tips of the long setae of its caudal rami touch the bottom. Should the specimen tilt, so as to descend to the bottom without touching it with the caudal setae, it will remain for some time motionless on the bottom. It might be presumed that the setae are equipped with a

sensory apparatus capable of registering and transmitting stimuli eliciting this reaction. This feature of the caligid behaviour might be associated with the movements of the adult parasite in the course of finding its host and settling on it.

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The Winter Flounder (*Pseudopleuronectes americanus*) in Long Pond, Conception Bay, Newfoundland[1,2,3]

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KENNEDY, V. S., AND D. H. STEELE. 1971. The winter flounder (*Pseudopleuronectes americanus*) in Long Pond, Conception Bay, Newfoundland. J. Fish. Res. Bd. Canada 28: 1153–1165.

Monthly samples of winter flounder taken in Long Pond from November 1962 to October 1963 indicated that the flounder moved into deeper water (7–10 m) during the summer and returned to shallow water (1–2 m) from September to June. These movements corresponded to the end of the spawning season and the ripening of the gonads respectively. Spawning occurred from March until early June, most of it in May and early June. Most males were mature at age 6 and most females at age 7. Fifty percent of the males and females were mature at 21 and 25 cm respectively. The growth rates of the males and females were similar until the age of 8, after which the females apparently outgrew the males. Early growth and fecundity were similar to those reported for other areas. No feeding took place in December or January but the flounder fed in March and continued to feed throughout the summer; food intake decreased in the fall. They were omnivorous and the type of food eaten varied with the locality. Polychaetes, plant material, and molluscs were the most common food items throughout the year. Capelin eggs and fish remains were found only during a few months of the year but were eaten in great quantities.

KENNEDY, V. S., AND D. H. STEELE. 1971. The winter flounder (*Pseudopleuronectes americanus*) in Long Pond, Conception Bay, Newfoundland. J. Fish. Res. Bd. Canada 28: 1153–1165.

Des échantillons mensuels de plie rouge ont été récoltés à Long Pond, à partir du mois de novembre 1962 jusqu'au mois d'octobre 1963. Ces derniers se déplacent vers l'eau profonde (7–10 m.) pendant l'été et retournent vers l'eau peu profonde (1–2 m.) à partir de septembre, où ils restent jusqu'au mois de juin. Le premier mouvement correspond avec la fin de la saison de frai et le dernier avec celle de la maturation des gonades. Le frai a lieu entre mars et début juin, ayant son maximum vers la fin de cette époque. La plupart des mâles sont mûrs a 6 ans, tandis que les femelles ne mûrissent qu'à l'âge de 7 ans. 50% des mâles mûrs mesurent 21 cm. et 50% des femelles mesurent 25 cm. Le degré de croissance des mâles et des femelles est identique jusqu'à l'âge de 8 ans; après cela les femelles surpassent les mâles. Première croissance et fécondité à Long Pond sont semblables à celles observées en d'autres endroits. La plie rouge ne prend pas de nourriture en décembre et janvier, mais commence à se nourrir en mars et continue à manger pendant tout l'été, la ration diminuant vers l'automne. Elle est omnivore et la composition de sa nourriture dépend de la localité. La nourriture se compose principalement de polychaetes, matière végétale et de mollusques. Quoique les oeufs de capelan et les résidus de poisson ne se trouvent que pendant quelques mois de l'année, durant ce temps ils sont consommés en grandes quantités.

Received May 11, 1967; revised August 14, 1970

MONTHLY samples of winter flounder, a common shallow-water species from Labrador to Georgia, were analysed in an effort to provide information on various aspects of the biology of a northern population. Four hundred and sixty flounder were collected in Long Pond, Conception Bay, Nfld., (Fig. 1) between November 1962 and October 1963 (Table 1). Because the flounder were absent from Long Pond in August, two samples were taken in that month from nearby Horse Cove, Conception Bay (Fig. 1).

[1]Based on a thesis submitted by V.S.K. in partial fulfillment of the requirements for the degree of Master of Science from Memorial University of Newfoundland.

[2]Studies in Biology from Memorial University of Newfoundland No. 239.

[3]Contribution from the Marine Sciences Research Laboratory No. 69.

[4]Present address: c/o R. M. Coffin, Port Republic, Maryland 20676, USA.

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Long Pond is a shallow (1–2 m) pond that opens into Conception Bay through a narrow gut, which has been dredged to an average depth of 6 or 7 m to form a small harbour at the mouth of the pond. Tides of 0.2–1.3-m amplitude flow freely into and out of the pond and maintain high salinities. The temperature in the deeper part of the pond does not differ markedly from that just outside the mouth.

Horse Cove is approximately 10 miles northeast of Long Pond and is only a small indentation in the coast line. Flounder were caught here at a depth of 7–10 m.

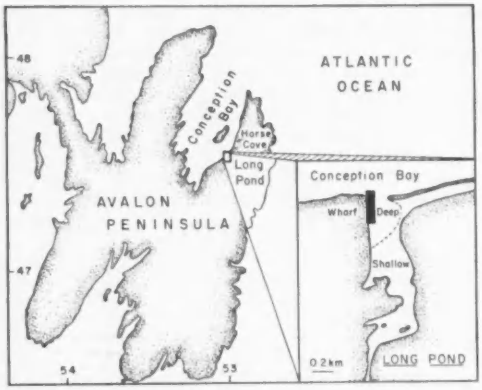

FIG. 1. The Avalon Penisula, Newfoundland, showing the locations of Long Pond and Horse Cove. Inset: Long Pond.

Materials and Methods

Details of the collections and environmental conditions are given in Table 1. An area of about 400 m² was spearfished on the later sampling trips.

The following information was recorded for each specimen. Total length was measured to the nearest centimeter from the tip of the mandible to the distal end of the longest caudal fin ray. Round weight was taken to the nearest gram. The gonads were examined visually and sex and maturity determined using criteria similar to those of Scott (1954). Both ovaries were removed from 23 females collected between December 1962 and May 1963 and were stored in modified Gilson's fluid (Simpson 1951). The numbers of eggs per fish were estimated following Pitt's method (1964). Ages were estimated from otolith readings. Age-groups were assigned according to the system of Kohler (1958). Stomach contents were analysed according to the point system (Ball 1961) and the relative importance of the organisms (identified to species) was determined by their frequencies of occurrence, numbers, and volumes.

Results

Distribution and Movements

Catches with gillnets between November and April demonstrated that some winter flounder were present in the shallow portion of Long Pond in this period (Table 1). Ice formation and theft of the nets hampered fishing in some months. The abundance of the fish could not be determined due to the different nets used.

From May to October, abundance was estimated by calculating the number of flounder caught per

TABLE 1. Monthly collections of winter flounder in Long Pond and Horse Cove from November 1962 to October 1963.

Month	Gear used	No. fish	Temp (C)	Salinity (‰)
		1962		
Nov.	2″, 4½″ gillnets	42	8.5	–
Dec.	2″, 4½″ gillnets	35	3–4	18.4
		1963		
Jan.	2″, 4½″ gillnets	5	–0.5	–
Mar.	2″ gillnet	37	0–1.2	–
Apr.	6″ gillnet	29	6	–
May	Scuba and spear	98	8	30.4
June	”	98	19.8–11	26.4–31.3
July	”	49	8–13.2	31.7–32.1
Aug.[a]	”	26	11.1	31.5–31.7
Sept.	”	28	10.6–11.2	31.2–31.6
Oct.	”	39	9.2	30.4

[a]Collections from Horse Cove; all others from Long Pond.

man-hour of diving (Table 2). Flounder were plentiful in the shallow area in May and decreased slightly in abundance through June. None were caught on July 8 in the shallows, although a few small specimens were seen around the edge of the dredged area. The bottom of the dredged area was searched on July 11 and many flounder of all sizes were observed, but in August few fish were noted. Many more were observed in the dredged area in September and by October flounder had returned to the shallow area.

Age and Growth

Hyaline edges were present throughout the year in otoliths of some Long Pond fish but the percentage of hyaline edges present declined from May to July. For all the fish collected in the various months, the percentages with hyaline edges were:

Month	Sample	No. fish with hyaline edges	% hyaline edges
Nov.	42	36	86
Dec.	35	34	97
Jan.	5	4	80
Feb.	0	–	–
Mar.	37	33	89
Apr.	29	26	90
May	98	55	56
June	98	30	31
July	49	2	4
Aug.[a]	26	20	77
Sept.	28	23	82
Oct.	39	35	90

[a]Sample from Horse Cove.

It would appear that, in agreement with Berry's (1959) findings for Rhode Island flounder, one hyaline and one opaque ring are added to the otoliths of Long Pond flounder each year.

For the sake of convenience, the data on size composition for some months were combined, but none from gillnet fishing with any from spear fishing. The fish caught during the year ranged in length from 12 to 46 cm and in age from 2 to 13 years (Fig. 2).

Fish caught during November and December ranged in length from 19 to 45 cm and in age from 4 to 11 years. In March, when only a 2-inch net was used, the size range was 18–26 cm (4–9 years), the catch being composed mainly of the 20–22-cm group (5 years). During April a 6-inch net was used and only large fish (28–44 cm, 6–12 years) were

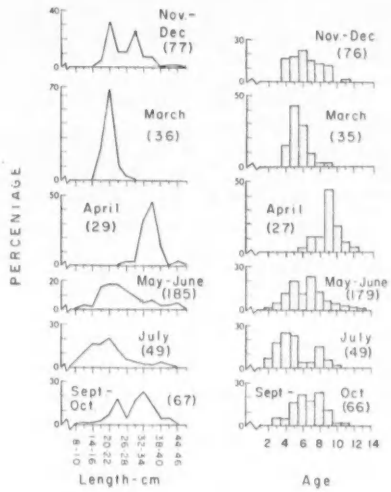

FIG. 2. Size and age compositions of catches of winter flounder from Long Pond. Numbers of fish in parentheses.

TABLE 2. Winter flounder catches per unit effort for Long Pond and Horse Cove, May–October 1963. S = surface; B = bottom.

Month	Depth (*m*)	Man-hr scuba	Catch/man-hr	Temp (*C*)
May	1–2	3	32.6	8(S)
June 12	1–2	3	20.0	9.8 (S)
June 28	1–2	2	14.5	11(S)
July 8	1–2	1	0.0	10–13.2(S)
July 11	5–7	3.5	14.0	8(B)
Aug.	5–7	2	0.0	11(B)
Aug.[a]	7–10	1.3	20.0	11.1(B)
Sept.	5–7	3	9.3	10.6–11.2(B)
Oct.	1–2	1.5	24.0	9.2(S)

[a]Horse Cove fished only on this occasion.

taken. Obviously mesh selection was an important factor during March and April.

Spearfishing during May and June took a wide range of flounder (12–46 cm, 2–13 years) with 17–28-cm fish most abundant (5–7 years of age). In July, fewer large old specimens were caught (they seemed to move out of Long Pond first) and the few fish seen in August in Long Pond were small. The flounder caught in Horse Cove in August were 15–37 cm long and 3–10 years old. Flounder reentering shallow water in September and October were 13–43 cm long and 3–11 years old.

For male and female flounder collected from May to July (Fig. 3), there was a close correspondence in mean lengths between the sexes until the age of 8, the females being generally slightly larger than the males. At the age of 9, females were much larger than males and the discrepancy increased with age. This may be due to the low numbers of females involved (9, 6, 4, 5, and 1 fish at ages 9, 10, 11, 12, and 13 respectively) or to failure to age the older fish correctly.

Regression equations of log weight against log length were calculated separately for males and females sampled during the April–June period:

female (117 fish) $\log W = 3.1441 \log L - 2.0702$;

male (107 fish) $\log W = 2.9833 \log L - 1.9041$.

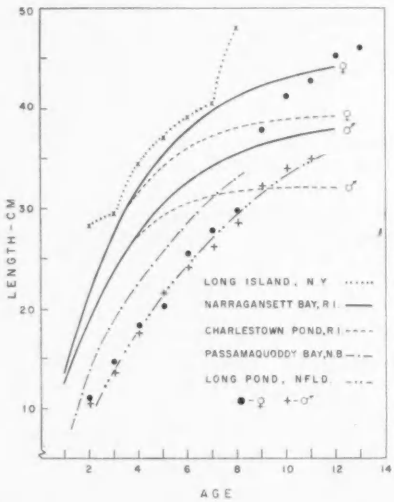

FIG. 3. Growth of winter flounder in Long Pond and southern areas. Long Pond data from collections during May to July. Long Island data from Perlmutter (1940). Fitted curves for Rhode Island data from Berry et al. (1965); line of best fit for Passamaquoddy Bay data estimated by eye from McCracken (1954).

As the difference in slope of the regression lines for males and females (Fig. 4A) was not statistically significant ($P > 0.05$), the two sexes were combined for further analysis.

The difference in weight between fish collected in July and September and those collected in April–June (Fig. 4B) was not statistically significant ($P > 0.05$).

REPRODUCTION

Analysis of age and size at maturity is based on data collected during the spawning period (March–early June). All individuals of both sexes were mature at the age of 9 but males tended to mature at a slightly younger age. The percentages of males and females taken from Long Pond from March to July that were mature at various ages were:

	Males		Females	
Age	No.	Mature (%)	No.	Mature (%)
3	2	0	6	0
4	8	40	18	0
5	23	43	28	10
6	15	87	17	35
7	28	87	19	74
8	14	95	14	90
9	11	100	13	100

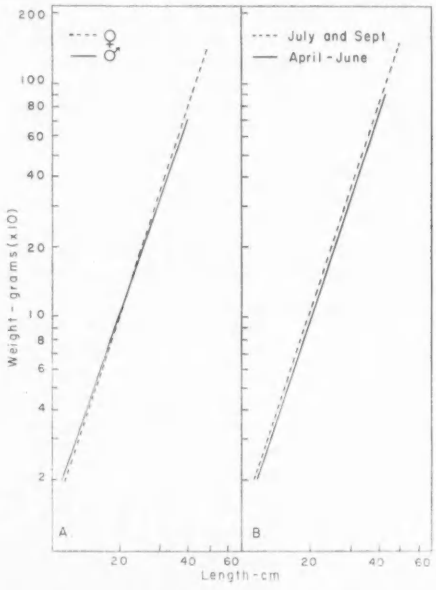

FIG. 4. Length–weight relations of round winter flounder from Long Pond, 1963. A, Males and females, April–June; B, combined data for males and females, April–June and July and September.

Size at maturity was estimated by probit analysis (Fleming 1960). Fifty percent of the males and females were estimated to mature at 21 and 25 cm respectively (Fig. 5).

The percentages of mature males and females in the various stages of the reproduction cycle (Fig. 6) indicate that when the survey began in November all the mature fish were ripening or (three of the males) already ripe. Spawning occurred from March to early June, most of it in May and early June.

In estimating fecundity, we calculated the percentage by which the estimate for each of three samples of eggs from each of the 23 fish deviated from their mean. This simple test indicated that the frequency estimates are consistent and reliable. The frequency distribution of the percentage deviations showed that 68 observations deviated from their means by less than 4% and one observation deviated by 4.4%:

Range of deviation (%)	No. observations deviating
0–1.9	50
2–3.9	18
4–5.0	1

Since fecundity was proportional to body size (Fig. 7, 8), the following equations were calculated:

$$\log F = -0.4517 + 4.0631 \log L$$

$$\log F = 2.3894 + 1.2403 \log W$$

where F = fecundity, L = length in centimeters, and W = weight in grams.

The values found in this study ranged from 99,000 eggs for a 22-cm fish weighing 111 g to 2,604,000 for a 44-cm fish weighing 1300 g. The mean was 590,000 eggs for a mean fish length of 34 cm.

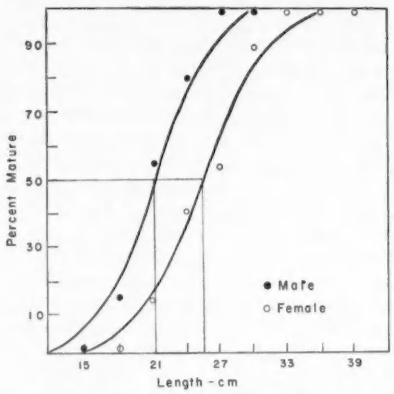

FIG. 5. Percentages of male and female winter flounder in Long Pond, March–June 1963, that were mature at various lengths.

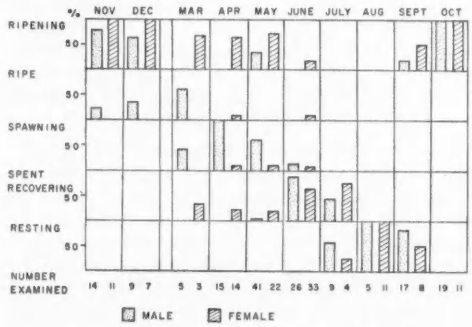

FIG. 6. Percentages of mature male and female winter flounder from Long Pond and Horse Cove (August sample), November 1962–October 1963, that were in various phases of the reproduction cycle.

Food and Feeding Habits

The most common food items of the flounder from Long Pond and Horse Cove were errant and sedentary polychaetes, pelecypods, fish eggs (in season), amphipods, copepods, fish remains, and plant material (Fig. 9A). The detailed tabulated results of the analysis are on file.

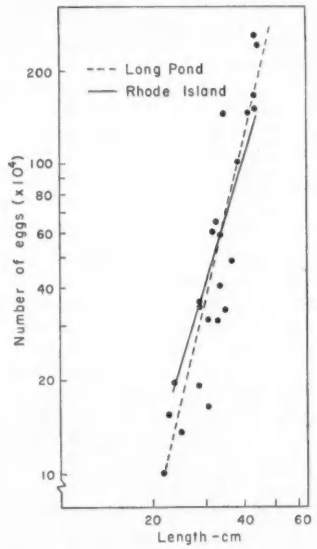

FIG. 7. Length–fecundity relations of winter flounder in Long Pond and Rhode Island (line of best fit for latter computed by least squares from data in Saila (1961b)). Plotted points are for individual Long Pond females, and the line was fitted by least squares.

The same items were found in all three length groups (8–19, 20–31, and 32–46 cm) into which the the flounder were arbitrarily divided, but there was a tendency for some, such as debris, polychaetes, winter flounder eggs, and small crustaceans, to decline with increasing size, whereas the amounts of fish remains and plant material increased (Fig. 9B). Capelin eggs were eaten in quantity by both the small and the large group, and molluscs were more important to the intermediate group than to the other two groups.

Seasonal variations in the amount of food eaten are shown in Fig. 10.

The fish fed from March till November (Fig. 10), and the variations in the amount of food eaten probably reflected the availability of various foods, such as capelin and eggs of winter flounder. However, there also seems to be a regular decline from September to December.

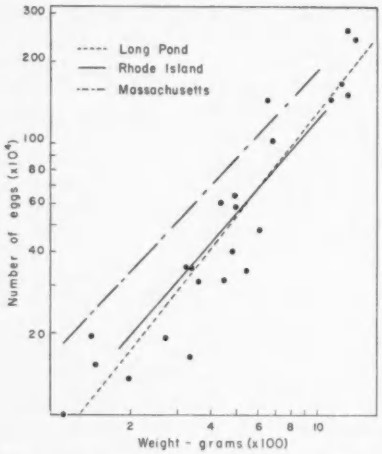

FIG. 8. Weight–fecundity relations of winter flounder in Long Pond, Rhode Island (Saila 1961b) and Massachusetts (Topp 1968). Plotted points are for individual Long Pond females.

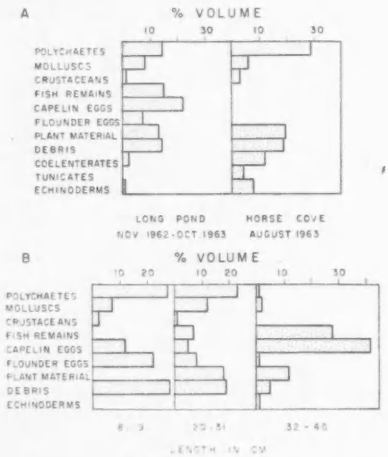

FIG. 9. Volume analyses of main food items in stomachs of winter flounder. A, From Long Pond, November 1962–October 1963, and Horse Cove, August 1963; B, from Long Pond only, according to three arbitrary size classes.

Discussion

Winter flounder appeared in Long Pond in the autumn, when the gonads were ripening; apparently remained until the spring, when they spawned; and left in the early summer, after spawning was completed. We have little information on their occurrence in Conception Bay outside of Long Pond, but their occurrence in shallow water at Horse Cove in the summer suggests that they probably remain in shallow water near Long Pond also.

McCracken (1963), in reviewing flounder movements on the Atlantic coast, concluded that two patterns of movement occurred: south of Cape Cod the movements are as described for Long Pond, whereas north of Cape Cod flounder move into deep water in the winter and remain inshore in the summer. These differences, he concluded, were due to differences in spawning seasons and water temperatures in the two regions and to the flounder having "a preferred temperature range of 12–15°C." However, the Long Pond flounder left before the temperature had reached these values

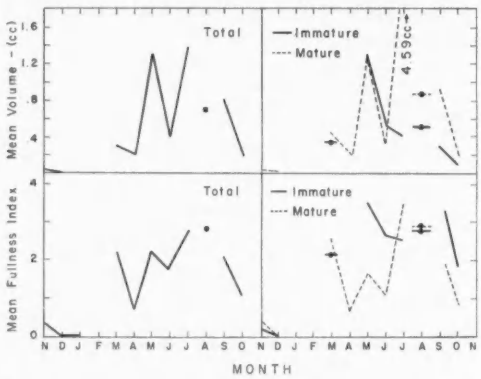

FIG. 10. Seasonal variations in food intake from November 1962–October 1963, for immature and mature flounder and for the two combined. The August data from Horse Cove are shown as solid circles.

and in August flounder were found outside, where the temperature was 11.1 C, but were absent inside, where the temperature was 11 C. This suggests that temperature preferences were of little significance in these movements. Study of the literature suggests, moreover, that the latitudinal differences in movements reported by McCracken (1963) may not exist. The areas where he reported that the flounder remain in shallow water in the summer are relatively open areas (Passamaquoddy Bay, St. Mary's Bay, and Northumberland Strait). Flounder are also found in the summer in shallow water in open areas of Long Island Sound (Lobell 1939) and Conception Bay (personal observations). However, flounder appear to leave estuaries (Pearcy 1962), harbours (McCracken 1963), and barachois ponds (Lobell 1939; present observations) in the summer throughout their range. This suggests that these summer movements are related to some factor other than a temperature preference. There is relatively little food in Long Pond (Christie 1966) and, if this is generally true of the above habitats, the summer movements may represent a feeding migration.

The annual growth increments for the Long Pond fish from 2 to 6 years of age are similar to those found in more southern populations even though the mean length at each age is less for the Long Pond flounder (Fig. 3). The greater mean lengths of the southern populations are presumably due to their earlier spawning period (see below) so that individuals are 2–6 months older than those in Long Pond.

Beyond the age of 6, Long Pond fish continue to grow without a marked decline, but to the south growth apparently slows abruptly so that Long Pond flounder are approximately the same length as their southern counterparts by the age of 10. The similarity in length may be due to (1) failure to age the older Long Pond fish correctly, (2) removal of the faster growing members of the southern populations by exploitation, or (3) the possibility that the southern individuals do grow more slowly at the older ages.

Berry (1959) presented a length–weight equation for 107 gonadless fish from Rhode Island, both sexes combined, which when expressed logarithmically is: $\log W = 3.01511 \log L - 3.0079$. This is similar to our findings though we did not remove the gonads before weighing the fish. Berry also notes that Saila (1959) found no statistically significant difference in the length–weight relations of male and female flounder, a fact in agreement with our determinations.

Bigelow and Schroeder (1953) thought that winter flounder in New York waters matured at age 3, since most of the spawners examined were longer than 8 inches (20.3 cm). This length at maturity is comparable to that estimated for Long Pond males although it is smaller than that estimated for Long Pond females. Maturity may be related to size rather than to age, with the northern flounder being older at maturity than the more southerly forms.

In Long Pond, the male gonads began to enlarge earlier than and reached the spawning stage before the female gonads. Although ripening in Long Pond began in September, it progressed slowly during the winter months so that spawning did not take place until from March to June. Comparison of the spawning season for the flounder in Long Pond with those in southern areas indicates that spawning generally occurs progressively earlier as one proceeds from north to south (Table 3).

There is no statistically significant difference between either the length–fecundity or the weight–fecundity curve estimated for the Long Pond samples and the corresponding curve calculated from the data of Saila (1961b, table 1) for 46 mature flounder from Rhode Island (Fig. 7, 8). The equations calculated from Saila's data were: $\log F = 0.8026 + 3.2507 \log L$, and $\log F = 2.6712 + 1.1383 \log W$. Topp (1968) obtained fecundity data from a sample of 30 Massachusetts winter flounder and arrived at the equation: $\log F = 0.0697 + 1.0659 \log W$. This equation is in error, and should be restated as: $\log F = 3.0697 + 1.0659 \log W$. The resulting regression fits the data in fig. 3 of Topp's paper (1968). Plotting the curve in Fig. 8 suggests a large difference between this and the Rhode Island and Newfoundland curves. Because of lack of the original data in Topp's paper, this difference was not tested statistically.

The estimates of fecundity in our study (from 99,000 eggs for a 22-cm, 111-g female to 2,604,000 eggs for a 44-cm, 1300-g female; average, 590,000 eggs for a mean fish length of 34 cm) are largely similar to those of other workers. Saila's estimates (1961b) range from 193,000 eggs (25-cm, 210-g fish) to 1,340,000 eggs (43-cm, 1052-g fish) with an average of 610,000 eggs for a mean fish length of 33.4 cm. Bigelow and Schroeder (1953) estimated the average fecundity of Gulf of Maine flounder to be 500,000 eggs with nearly 1,500,000 eggs having been taken from a 3¾-lb (1531-g) female. Topp's range is 435,000 eggs from an age 3 fish to 3,329,000 eggs from an age 5 fish. Figure 1 of his paper indicates that his 3-year-old fish ranged in size from 30 to 40 cm, and 5-year-olds were 40–45 cm long. Judging from the data in fig. 3 of his paper, the flounder producing 435,000 eggs weighed about 300 g, whereas the fish producing over 3 million

TABLE 3. Spawning seasons for winter flounder from different areas arranged from south to north.

Region	Range of spawning season	Maximum spawning	Authority
S New England and New York	Mid-Dec.–May	Varies with area	Perlmutter 1939, 1947
S New England	Feb.–Apr.	–	Tracy 1910; Smith 1898
S New England	Jan.–Mar.	–	Bigelow & Welsh 1925
Long Island	Dec. 15–May 30	–	Lobell 1939
Mystic River, Conn.	Mid-Feb.–Apr.	Mar.	Pearcy 1962
Green Hill Pond, R.I.	Dec.–Mar.	–	Saila 1961a
Narragansett Bay, R.I.	Larvae found Feb.–June (hatch 15–18 days after spawning)	–	Herman 1963
Cape Cod South	Jan.–May	Feb.–Mar.	Bigelow & Schroeder 1953
Boothbay, Me.	Mar. 1–May 5	Mar. 30–Apr. 20	Bigelow & Schroeder 1953
Passamaquoddy Bay, N.B.	–	Late Apr.–early May	McCracken (personal communication
St. Mary's Bay, N.S.	Seems to be earlier than for Passamaquoddy Bay	Many spent in late Apr.	McCracken (personal communication
Long Pond, Nfld.	Mar. ?–early June	May–early June	

eggs weighed about 1400 g. Using the regression equations given above, a 1000-g female would be expected to produce 1,219,000 eggs in Rhode Island, 1,851,000 eggs in Massachusetts, and 1,288,000 eggs in Newfoundland. The reported differences in fecundity estimates are probably attributable in part to differences in technique.

The winter flounder in both Long Pond and Horse Cove were omnivorous, a situation previously noted for Woods Hole (Linton 1921) and Long Island Sound (Richards 1963) populations. Differences in diet in Newfoundland populations can be ascribed to differences in prey availability. The bottom at Long Pond is mostly muddy sand, whereas Horse Cove has a bottom of coarser sand with less mud and with more exposed rocks and boulders. Consequently, differences in the bottom fauna would be expected. The importance of food availability is reflected in the gorging of mature flounder on capelin eggs in July. Tibbo et al. (1963) reported *P. americanus* preying heavily on herring eggs in the Bay of Chaleur. In Long Island South, Richards (1963) found that "seasonal changes in the type of prey consumed were due in part to availability of the latter..."

Both infauna and epifauna figure in the diet. The presence of *Pectinaria gouldi* in their tubes and quantities of infaunal molluscs, sand, and debris in stomachs indicates that the flounder dig in the bottom on occasion. Richards (1963) indicated that all polychaetes, whether errant or sedentary, were liable to be eaten by young winter flounder. Amphipods in waters to the south of Newfoundland (Linton 1921; Smith 1950; Richards 1963) were a more important food item than in Long Pond or Horse Cove. Molluscs have also been noted as important in the diet (Bigelow and Schroeder 1953; Richards 1963). Hydroids and algae were utilised but this may have been incidental to the consumption of associated encrusting or clinging prey. Linton (1921) felt that young flounder whose stomachs had been found full of plant material had been actually attracted by an encrusting tunicate. F. D. McCracken (personal communication) noted green algae in 50% of the flounder caught inshore during May and June, with a decrease to 10% during July and August. Whether plant material is ingested for its intrinsic food value remains to be clarified.

The broad food groups in the diet of 8–19-cm fish noted in this study are similar to those eaten by 7.6–22.5-cm flounder taken at Woods Hole in the summers of 1915 and 1916 (Linton 1921) and by 3.7–16.5-cm animals from Long Island Sound (Richards 1963). These southern specimens ate amphipods and other small crustaceans, polychaetes, molluscs, and hydroids along with debris and some plant material. No fish eggs were noted.

Feeding occurred during spring and summer and then tapered off during the autumn, with no food present in the stomachs in winter. Richards (1963) noted that empty stomachs occurred in juvenile (3.7–16.5-cm) flounder only in the winter and early spring. We have no information on the direct influence of decreasing temperature on winter flounder feeding activity, so the reasons for a winter feeding slump are obscure. In addition, our samples were not taken during any set time of day or at any constant interval in a tidal cycle. Consequently, if winter flounder are stimulated to feed by such

extrinsic factors as photoperiod or state of the tide, our assessments of feeding intensity and volume of food eaten might be affected.

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(APPENDIX TABLES 1–4 *follow*)

APPENDIX TABLE 1. Stomach content analysis, Long Pond, November 1962–October 1963.

Food organisms	No. organisms	Stomachs in which occurred[a]		Aggregate total vol[b]	
		No.	%	cc	%
ANNELIDA		159	62.5	39.45	17.2
POLYCHAETA		159	62.5	32.85	14.3
Polychaeta Errantia	419	128	50.3	23.59	10.3
Anaitides sp.	94	40	15.7	0.54	0.2
Eteone sp.	202	64	25.1	1.89	0.8
Nereis pelagica	32	25	9.8	10.87	4.7
Nereis virens	15	12	4.7	7.15	3.1
Eulalia sp.	1	1	0.3	0.01	0.0
Polynoidae	75	20	7.9	0.58	0.2
Unidentified Nereids	–	19	7.4	1.42	0.6
Unidentified Errantia	–	40	15.7	1.13	0.4
Polychaeta Sedentaria		96	37.7	9.26	4.0
Pectinaria gouldi	235	35	13.7	2.38	1.0
Arenicola marina	418	10	3.9	2.37	1.0
Polydora (*gracilis*?)	2	2	0.7	0.01	0.0
Orbiniidae (*Scoloplos sp.*?)	77	29	11.4	0.26	0.1
Cirratulidae	3	2	0.7	0.01	0.0
Sabellidae	1	1	0.3	0.01	0.0
Maldanidae	2	2	0.7	0.01	0.0
Unidentified Sedentaria	–	60	23.6	4.21	1.8
GEPHYREA					
Echiurida					
Echiurus sp.	1	1	0.3	6.60	2.9
ARTHROPODA		123	48.4	2.84	1.2
CRUSTACEA		123	48.4	2.77	1.2
Copepoda					
Harpacticoida	5017	71	28.0	0.88	0.4
Cirripedia					
Balanus balanoides	1	1	0.3	0.02	0.0
Cumacea	13	13	5.1	0.05	0.0
Amphipoda	401	76	29.9	1.32	0.6
Gammaridea	395	76	29.9	1.29	0.6
Orchomenella minuta		14	5.5		
Phoxocephalus holbolli		4	1.5		
Monoculodes intermedius?		9	3.5		
Metopa sp.		7	2.7		
Mesometopa neglecta		1	0.3		
Calliopius laeviusculus		9	3.5		
Sympleustes glaber		2	0.7		
Pontogeneia inermis		4	1.5		
Dexamine spinosa		20	7.9		
Gammarus oceanicus		14	5.5		
Gammarus lawrencianus		3	1.1		
Leptocheirus pinquis		1	0.3		
Photis reinhardi		7	2.7		
Corophium bonelli		26	10.2		
Ischyrocerus anquipes		15	5.9		
Caprellidea	6	2	0.7	0.03	0.0
Caprella septentrionalis		2	0.7		
Decapoda					
Crangon septemspinosus	1	1	0.3		
Pagurus sp.	1	1	0.3	0.40	0.2
Cancer irroratus	1	1	0.3	0.10	0.0
INSECTA					
Chironomid larvae	13	10	3.8	0.06	0.0
PYCNOGONIDA	3	3	1.1	0.01	0.0

(*Continued*)

APPENDIX TABLE 1. Stomach content analysis, Long Pond, November 1962–October 1963. (*Concluded*)

Food organisms	No. organisms	Stomachs in which occurred[a]		Aggregate total vol[b]	
		No.	%	cc	%
MOLLUSCA		193	75.9	15.64	6.8
AMPHINEURA					
Ischnochiton ruber	17	1	0.3	0.01	0.0
GASTROPODA		78	30.7	1.86	0.8
Opisthobranchiata					
Nudibranchiata	7	3	1.1	0.04	0.0
Prosobranchiata					
Acmaea testudinalis	19	5	1.9	0.40	0.2
Puncturella noachina	1	1	0.3	0.01	0.0
Hydrobia minuta	84	38	14.9	0.26	0.1
Littorina sp.	65	38	14.9	0.50	0.2
Lacuna vincta	22	9	3.5	0.30	0.1
Lacuna pallidula neritoidea	1	1	0.3	0.01	0.0
Margarites helicinus	1	1	0.3	0.01	0.0
Buccinum undatum	1	1	0.3	0.25	0.1
Lunatia heros	1	1	0.3	0.07	0.0
Unidentified Gastropods	3	2	0.7	0.01	0.0
PELECYPODA		177	69.6	13.38	5.8
Mytilus edulis	453	78	30.7	0.36	0.2
Crenella faba	7	7	2.7	0.04	0.0
Serripes groenlandicus	1	1	0.3	0.08	0.0
Hiatella arctica	85	3	1.1	0.04	0.0
Macoma balthica	2478	101	39.7	10.39	4.6
Mya arenaria	156	55	21.6	2.24	1.0
Mya truncata?	19	8	3.1	0.06	0.0
Cerastoderma pinnulatum	6	5	1.9	0.10	0.0
Clinocardium ciliatum	1	1	0.3	0.01	0.0
Unidentified Pelecypods		2	0.7	0.04	0.0
Siphons	5	5	1.9	0.39	0.2
ECHINODERMATA		11	4.3	1.20	0.5
Strongylocentrotus droebachiensis	17	7	2.7	0.86	0.4
Asterias vulgaris	7	2	0.7	0.30	0.1
Echinarachnius parma	4	2	0.7	0.03	0.0
Amphiuridae	1	1	0.3	0.01	0.0
COELENTERATA		9	3.5	4.25	1.8
HYDROZOA					
Campanularia		6	2.3	0.23	0.1
ANTHOZOA					
Actinaria		4	1.5	4.02	1.8
ECTOPROCTA					
Bugula sp.		1	0.3	1.21	0.5
CHORDATA					
ASCIDIACEA	6	4	1.5	0.05	0.0
PISCES					
Fish remains		11	4.3	35.06	15.3
Eggs — flounder		32	12.5	16.63	7.2
— capelin		15	5.9	51.05	22.2
— lumpfish		1	0.3	0.01	0.0
— unidentified				0.08	0.0
Miscellaneous					
Plant material		61	24.0	29.04	12.6
Debris (sand, stones, dead plants)		202	79.5	33.03	14.4

[a]Number of stomachs containing food, 254.
[b]Total volume, 229.54 cc.

APPENDIX TABLE 2. Stomach content analysis, Horse Cove, August 1963.

Food organisms	No. organisms	Stomachs in which occurred[a]		Aggregate total vol[b]	
		N	%	cc	%
ANNELIDA		23	92	5.42	28.9
Polychaeta Errantia		22	88	0.70	3.7
Anaitides sp.	20	11	44	0.06	0.3
Eteone arctica	11	9	36	0.07	0.4
Eteone trilineata	1	1	4	0.01	0.0
Nereis sp.	13	5	20	0.18	1.0
Eusyllis tubifex	15	8	32	0.05	0.3
Polynoidae	40	16	64	0.30	1.6
Unidentified Errantia		4	16	0.03	0.2
Polychaeta Sedentaria		22	88	4.72	25.1
Pectinaria gouldi	22	7	28	0.12	0.6
Arenicola marina	15	5	20	3.41	18.1
Amphitrite sp.	23	5	20	0.12	0.6
Ophelia radiata	1	1	4	0.18	1.0
Orbiniidae (*Scoloplos* sp.?)	22	8	32	0.11	0.6
Cirratulidae	49	17	68	0.13	0.7
Unidentified Sedentaria		21	84	0.65	3.5
ARTHROPODA		19	76	0.55	2.9
CRUSTACEA		19	76	0.55	2.9
Copepoda — Harpacticoida	62	10	40	0.02	0.1
Amphipoda	252	19	76	0.53	2.8
Gammaridea	204	19	76	0.47	2.5
Phoxocephalus holbolli		8	32		
Monoculodes intermedius?		3	12		
Metopa sp.		3	12		
Metopella carinata		1	4		
Calliopius laeviusculus		1	4		
Sympleustes glaber		1	4		
Parapleustes sp.		4	16		
Pontogeneia inermis		1	4		
Dexamine spinosa		5	20		
Gammarus oceanicus		1	4		
Corophium bonelli		12	48		
Unicola leucopis		4	16		
Ischyrocerus anguipes		5	20		
Jassa falcata		2	8		
Caprellidea	48	3	12	0.06	0.3
Caprella septentrionalis		2	8		
MOLLUSCA		16	64	1.15	6.1
Ischnochiton ruber	17	8	32	0.51	2.7
GASTROPODA		5	20	0.29	1.5
Acmaea testudinalis	10	7	28	0.25	1.3
Margarites helicinus	3	3	12	0.01	0.0
Littorina sp.	11	2	8	0.01	0.0
Unidentified Gastropods	1	1	4	0.01	0.0
PELECYPODA		7	28	0.35	1.9
Mytilidae (*Mytilus edulis* and *Volsella modiolus*)	548	4	16	0.30	1.6
Crenella faba	2	2	8	0.02	0.1
Mya arenaria	2	2	4	0.01	0.1
Mya truncata?	2	2	8	0.01	0.0
Unidentified Mollusca		1	4	0.01	0.0
ECHINODERMATA		22	88	1.46	7.8
Strongylocentrotus droebachiensis	98	16	64	0.29	1.5
Asterias vulgaris	43	9	36	0.45	2.4
Amphiuridae	19	14	56	0.72	3.8

(*Continued*)

APPENDIX TABLE 2. Stomach content analysis, Horses Cove, August 1863. (*Concluded*)

Food organisms	No. organisms	Stomachs in which occurred[a]		Aggregate total vol[b]	
		N	%	cc	%
COELENTERATA		8	32	2.26	12.0
Campanularia	1	1	4	0.07	0.4
Actinaria		7	28	2.19	11.6
CHORDATA	47	18	72	0.72	3.8
ASCIDIACEA	47	18	72	0.72	3.8
Miscellaneous					
Fly	1	1	4	0.02	0.1
Gastropod egg case	1	1	4	0.03	0.2
Plant material		11	44	3.60	19.2
Debris (sand, stones, etc.)		18	72	3.54	18.9

[a]Number of stomachs containing food, 25.
[b]Total volume, 18.75 cc.

APPENDIX TABLE 3. Percentage volume per month of the main food groups eaten by winter flounder in Long Pond, November 1962–October 1963.

Food group	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
Polychaeta	44	0	0	–	73	3	14	19	5	–	9	6
Errantia	43	0	0	–	67	3	14	9	0.6	–	0.5	2
Sedentaria	1	0	0	–	4	0.3	0	9	4	–	8	4
Mollusca	24	0	0	–	2	20	2	22	2	–	4	28
Pelecypoda	22	0	0	–	2	14	2	21	2	–	2	27
Gastropoda	2	0	0	–	0.3	6	0.1	0.7	0.1	–	2	0.9
Crustacea	9	0	0	–	2	0.3	0.8	0.3	0.4	–	1	1
Copepoda	0	0	0	–	0	0.1	0.8	0.3	0.1	–	0	0.1
Echinodermata	0	0	0	–	0	0	0	0.1	0	–	5	0
Winter flounder eggs	0	0	0	–	0	0	19	9	0	–	0	0
Capelin eggs	0	0	0	–	0	0	0	0	76	–	0	0
Fish	0	0	0	–	0	0	40	17	0.2	–	0.4	0
Plant material	4	0	0	–	5	57	6	9	10	–	31	49
Debris	18	100	0	–	17	15	20	25	6	–	2	17

APPENDIX TABLE 4. Percentage frequency of occurrence per month of the main food groups eaten by winter flounder in Long Pond, November 1962–October 1963.

Food group	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
Polychaeta	46	0	0	–	97	40	51	58	61	–	90	54
Errantia	23	0	0	–	97	30	44	44	54	–	48	33
Sedentaria	23	0	0	–	50	30	21	35	45	–	90	46
Mollusca	79	0	0	–	28	100	86	82	77	–	81	83
Pelecypoda	69	0	0	–	25	70	79	79	77	–	57	83
Gastropoda	23	0	0	–	6	60	40	36	25	–	38	25
Crustacea	8	0	0	–	22	20	16	24	48	–	71	25
Copepoda	0	0	0	–	0	30	67	27	50	–	57	13
Echinodermata	0	0	0	–	0	0	0	2	7	–	38	0
Winter flounder eggs	0	0	0	–	0	0	46	17	0	–	0	0
Capelin eggs	0	0	0	–	0	0	0	0	36	–	0	0
Fish	0	0	0	–	0	0	5	11	2	–	5	0
Plant material	8	0	0	–	25	50	16	12	30	–	57	25
Debris	92	100	0	–	84	70	98	82	82	–	62	63

Net Avoidance Behavior in American Shad (*Alosa sapidissima*) as Observed by Ultrasonic Tracking Techniques

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LEGGETT, W. C., AND R. A. JONES. 1971. Net avoidance behavior in American shad (*Alosa sapidissima*) as observed by ultrasonic tracking techniques. J. Fish. Res. Bd. Canada 28: 1167–1171.

During tracking of 13 adult American shad (*Alosa sapidissima*) with ultrasonic transmitters in 49 approaches to commercial drift gillnets in the lower Connecticut River, only one shad was captured. Typically, shad moved to within 1–2 m of the net before sensing its presence, then turned and swam along the net, close to the mesh, to its end, where they turned and continued their upriver migration. Sight appeared to play an important role in net detection. However, 14 avoidances were observed when light intensities were inadequate to allow visual detection of the mesh. It was concluded that other senses, perhaps the lateral line, also function in net avoidance, especially when sight is impaired.

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IN this study, the responses of adult American shad (*Alosa sapidissima* (Wilson)) to commercial drift gillnets, which they encountered during their upriver spawning migrations in the Connecticut River, were investigated. In the Connecticut River, the commercial shad fishery utilizes drift gillnets almost exclusively. These nets are 250–400 m long and are generally tailored to the area fished so that they reach close to the bottom. Approximately 40–50 drift nets are licensed by the state for fishing each year. All nets are drifted in the lower 56 km of the river. Fishing is allowed five nights per week, and each net is drifted several times each night. These combined factors result in a heavy fishing pressure on the species. Additionally, the upstream migration of shad is largely channel-oriented (Leggett 1967; Leggett and Jones 1968), which results in maximum probability of contact between migrating shad and the nets. However, in spite of heavy fishing pressure and this favourable behavior pattern, the commercial fishery has removed only 11–21% of the available shad migrating in the river annually since 1965 (W. C. Leggett unpublished data).

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Materials and Methods

During 1967, 1968, and 1969, 13 shad were captured with monofilament gillnets having identical mesh dimensions to those employed in the commercial fishery. Each shad was removed from the net immediately upon capture, as indicated by movement of the headrope floats, and an ultrasonic transmitter was inserted into the stomach via the mouth. The fish was then gently returned to the water. In all instances the elapsed time from capture to release was less than 1 min.

Transmitters used in 1967 produced an ultrasonic pulse at a frequency of 160 kHz; in 1968 and 1969, 70-kHz transmitters were used. Effective signal range for both types was 100 m–1 km depending on flow, turbidity, and other conditions in the river. The 70 kHz units were more effective under conditions where ambient noise was greatest. The transmitters were housed in polystyrene cylinders 6.4 cm long and 1.3 cm in diameter; their approximate weight was 85 g in air; and their effective life was 2–3 weeks.

Receiving equipment consisted of a portable, battery-operated receiver, tunable in the range 60–180 kHz, which was coupled to a hydrophone having peak sensitivity at 70 kHz. The hydrophone was constructed with a cone of reception of approximately 15°, and was mounted on a 5-m outboard runabout in a manner that allowed it to be rotated through 360°.

Tracking procedures included aligning the boat directly behind the migrating shad, matching boat speed to fish speed, and estimating fish location by changes in incoming signal strength. As the boat approached the

fish, signal strength increased and changed dramatically as the hydrophone passed over the fish, thereby allowing precise locating. When commercial nets were approached by the migrating shad, the tracking boat was maneuvered to a position lateral to the fish to estimate its proximity to the net. On two occasions the boat was anchored near the river edge and observations of net avoidance were made from there.

Fish movements and net locations were recorded on enlargements of the navigation charts of the U.S. Coast and Geodetic Survey. River velocity was measured periodically with a Gurley direct-reading flowmeter. Water temperatures were measured to the nearest half degree with an electronic thermometer.

The Connecticut River is tidal in the areas where net avoidance observations were made; shad were observed to approach nets during all stages of the tidal cycle, that is, ebb, slack, and flood. Approaches were also observed during daylight, twilight, and dark.

Results

Shad were observed during 49 approaches to commercial gillnets. The details of these approaches, together with data on prevailing conditions, are summarized in Table 1. Definite avoidance behavior was recorded on 42 of the approaches. On another six approaches, one shad passed within 5–20 m of the end of the nets without exhibiting any avoidance reaction. One shad (no. 1, Table 1) passed directly through, or under, the net without hesitation. On 40 of the 42 net avoidances, behavior was essentially identical: each shad swam directly toward the net until it was within .5–1 m of the mesh; at this point each fish turned and swam along the net, maintaining close proximity to the mesh; on reaching the end of the net, the shad turned abruptly and continued its upstream migration. On seven occasions, fish encountered a second net and avoided it in the same way; five of these avoided three or more nets (Fig. 1). There was no consistency in the path taken in avoiding nets; some shad approached nets near one end and swam to the opposite end to clear; others rounded the near end. Fish approaching the nets near their center also moved left and right without preference. No deviation in behavior was noted with respect to the state of the tide. In all instances, the shad, once having sensed the net, remained within a very short distance of the mesh until it reached the end. Behavior was identical during daylight, dusk, and darkness. On the remaining two avoidances the shad (no. 8 and 13, Table 1) behaved in a different manner. These fish, on detecting the net, turned downstream for 60–100 m. One track was terminated here because of navigational hazards that arose when fog developed. The second fish made a lateral movement that carried it beyond the reach of the net when it resumed its upstream migration. Only one of the 13 shad tracked (no. 12, Table 1) was captured by a commercial net while being observed.

Discussion

This study has shown that adult shad have a remarkable ability to detect and avoid commercial drift gillnets. This ability has long been suspected by commercial fishermen, who find their efforts more successful at night and largely restrict their fishing to the dark hours. In the past, monofilament nets proved very successful during daylight, and these have now been outlawed for commercial use.

The improved catches gained by fishing in relative darkness, and the greater efficiency of monofilament nets suggest that, in shad, sight plays an important role in net detection. This is consistent with the findings of Parrish (1969), who concluded, on the basis of a review of experimental studies of fish reactions to stationary and moving objects, that visual stimuli appear to be the main clues influencing the behavior of fish in the vicinity of fishing gear.

The close approach of shad to the net during avoidance could result from limited visibility of the mesh. The Connecticut River is turbid in May. Secchi disk readings in May, over a number of years, have ranged from 1 to 2 m. Hester and Taylor (1965) showed that the range of visual detection of bunched netting, which they considered to be similar in optical properties to a secchi disk, was 11–26% greater than that of open net meshes. Thus, visual detection of gillnets by shad during daylight was probably limited to less than 2 m.

Thirty-five net avoidances were observed at night when the range of visual detection of the nets would be further reduced or perhaps eliminated. Whitney (1969) summarized the light intensities at which several fish species stopped schooling, presumably because of loss of visual clues. For Atlantic herring, *Clupea harengus harengus*, the closest relative to shad among the species reviewed, the light intensities at which schooling behavior ceased ranged between 10^{-3} lx and 1 lx, depending on the experimental method employed. Only one of the eight marine species considered was able to maintain schooling behavior at light intensities below 1.7×10^{-6} lx. Blaxter and Parrish (1965), working with *C. harengus harengus*, showed that more light is required to see a net than to maintain schooling. They observed a reduction in the efficiency with which herring avoided nets when

TABLE 1. Summary of net avoidance behavior and associated data (meteorological data from U.S. Weather Office, Hartford, Connecticut).

Shad	Date	Time (EST)	Sky % overcast	Moon phase	Moon up	Current dirn. & velocity (*m/sec*)	No. approaches	Description of behavior
					1967			
1	May 2	3:00 AM	100	1 day after last quarter	Yes	Flood 0.76	1	Passed through center of net (under?) without hesitation.
2	3	12:01 AM–4:30 AM	100	2 days after last quarter	Rose 0226	Ebb to slack 0.76–0.0	12	Maintained one position, moved laterally to avoid nets, and returned to approximated former position.
3	24	7:00 PM–9:10 PM	100	Full	Rose 2040	Ebb 0.76	2	Passed directly by first net with a slight avoidance of the end, approached second net in center and swam laterally to end and around.
4	31	9:00 PM	60	1 day after last quarter	No	Ebb 0.15	1	Approached net near end, turned and swam full length to opposite end and around.
					1968			
5	May 9	7:40 PM	100	4 days after first quarter	Yes	Flood 0.21	1	Approached net at approximate center, swam to far end and around.
6	10	2:45 AM–4:00 AM	80–90	5 days after first quarter	Set 0312	Ebb 0.21–1.07	12	Maintained stationary position, moving laterally to end of nets and returning to original position after net passed by.
7	13	8:10 PM–9:40 PM	0	1 day after full	Yes	Ebb 0.18 to flood 0.09	6	Approached 3 nets near center and moved laterally, 4th net approached near end, moved slightly laterally, 2 nets passed closely but no overt avoidance behavior recorded.
8	14	12:30 PM	0	2 days after full	Yes	Ebb 0.15	1	Net blocked river, fish approached center, turned, and moved approximately 100 m downstream, fish then lost in fog.
9	16	7:15 PM–7:45 PM	100	4 days after full	No.	Ebb 0.15	3	Passed first net near one end with slight lateral movement. Second and third net approached near center, with long lateral movement to end and around.
					1969			
10	May 13	12:30 PM	80	3 days before full	Yes	Ebb 0.15	1	Approached net at center, turned and moved laterally to end and around.
11	19	9:40 PM–11:50 PM	100	3 days after full	Set 2238	Ebb 0.04	6	Passed nets 1 and 2 near one end with little if any change in course. Nets 3, 4, 5, approached near center and avoided laterally. Net 6 passed without apparent detection.
12	22	12:15 AM–12:25 AM	50	5 days after full	No	Slack 0.0	2	Passed end of first net with slight avoidance; approached second at one end, moved laterally approximately half way to opposite end and was then caught.
13	22	10:30 PM	100	5 days after full	Yes	Ebb 0.61	1	Approached net at center, moved laterally, then turned downstream, approximately 60 m, again moved laterally and upstream past net without apparent detection.

light intensities dropped below 6×10^{-3} lx. This was true for both field and laboratory studies.

Unfortunately, we have no precise information on light intensities at various depths in the Connecticut River at the time of our observations. However, it was possible to estimate the amount of light that was available during several of the avoidances, and these data strongly suggest that shad were able to detect gillnets when light intensities were too low to allow visual detection of the twine. Surface light intensities resulting from star and skylight, and full moon, approximate 5.4×10^{-4} and 5.4×10^{-1} lx, respectively, and may be reduced by 50–90% depending on cloud conditions (Brown 1952). In the water, light intensities decrease rapidly with depth. Tyler (1968) related secchi disc readings to light intensities at equal depths. In ocean water, with a secchi disc reading of 2 m, such as we obtained in the Connecticut River, 90% of the surface light was absorbed in the top 1–2 m. In the more turbid Connecticut River, light loss with depth would be greater.

Three shad (no. 4, 11, 12; Table 1) avoided nets on moonless nights when cloud cover ranged

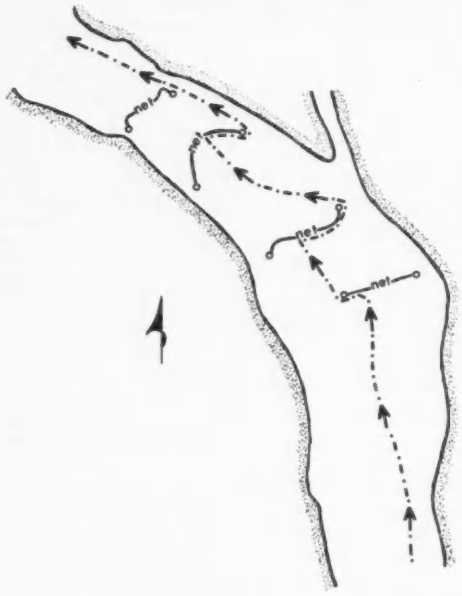

FIG. 1. Net avoidance behavior of shad no. 11, Table 1, at nets 3, 4, 5, and 6.

from 50 to 100%. Ignoring the influence of cloud, surface light intensities at the time of our observations would be approximately 5.4×10^{-4} lx; at 2 m depth the intensity would be approximately 5.4×10^{-6} lx. These estimates are well below the critical levels reported by Whitney (1969) and Blaxter and Parrish (1965). Three additional shad (no. 1, 2, 13; Table 1) avoided nets when cloud cover was complete and the moon was less than full. We assumed there was a full moon to provide a margin of safety in our calculations, and reduced the surface intensity by 50% to correct for the influence of cloud cover. We estimated a surface intensity of 2.7×10^{-2} lx, which gave a maximum intensity of 2.7×10^{-4} lx at 2 m, again below the critical levels described above. It should be noted that only five avoidances attributed to shad no. 2 (Table 1) meet these criteria. The remainder occurred during twilight.

If vision was eliminated at such times, how were shad able to detect the nets? One possible alternative to vision might be the sensing of low frequency hydroacoustic sources generated by the movement of water through the twine. It is highly probable that such hydroacoustic stimuli would be set up around the meshes of drifting gillnets by the shears and vertical and horizontal turbulence that is characteristic of the Connecticut River, since these would act to create nonuniform velocities along the twine. It is well established that fish are able to detect such hydroacoustic stimuli, generated by fish and other obstacles, by means of their lateral-line receptors (Fitzgerald 1967). The degree to which this ability is developed varies greatly among the various species of fish, and only a few species are able to locate stationary objects in their path (Kuiper 1967). Several workers (John 1957; Kuiper 1967; Dijkgraaf 1967), in discussing the ability of blind or blinded fish to detect stationary objects, have stressed the importance of eliminating all externally introduced vibrations that could serve to alert the fish to the obstacle. These authors ascribe the detection of such vibrational stimuli to the lateral line system. Such stimuli, if detected by shad not able to use their eyes to full advantage because of light conditions, might aid in warning them of the presence of the net and in guiding their avoidance movements. Whatever the mechanism for avoidance, the observed remarkable ability of shad to avoid nets helps to explain the observed low probability of capture of shad in commercial gillnets fished in the Connecticut River.

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Levels of Constituents of Glycoproteins in the Sera of Pacific Salmon[1]

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QURESHI, M. D., R. V. HLEDIN, W. E. VANSTONE, AND P. A. ANASTASSIADIS. 1971. Levels of constituents of glycoproteins in the sera of Pacific salmon. J. Fish. Res. Bd. Canada 28: 1173–1179.

The levels of hexosamine, sialic acid, fucose, and protein in serum of sockeye salmon (*Oncorhynchus nerka*) and, to a limited extent, in sera of coho salmon (*O. kisutch*) and chinook salmon (*O. tshawytscha*) at two reproductive stages, were determined. Hexosamine, sialic acid, fucose, hexose, seromucoid, and protein content of sexually maturing (early) and mature (spawning) sockeye salmon were studied and a comparison was attempted with the corresponding composition of bovine serum. Content of the above serum constituents was lower in spawning than in maturing populations. Protein content was much less, hexosamine a little less, and sialic acid higher, in the sera of sockeye salmon than in bovine serum. The protein–carbohydrate complex of serum appeared to contain more hexosamine and much more sialic acid than the protein–carbohydrate complex of bovine serum. Furthermore, the sialic acid-to-hexosamine ratio was much higher in sera of salmon than in bovine serum. Some other sex and reproductive stage differences were detected and reported.

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IN view of the great productive and nutritional significance of salmon, an investigation was started in 1964 to determine the composition of blood sera of some species of salmon in the characteristic glycoprotein constituents and the electrophoretic patterns of these sera, and to isolate and characterize different glycoproteins of the plasma. This paper reports on the results of this work on the composition of the sera in glycoprotein constituents, and a subsequent paper will report on the resolution of glycoproteins by electrophoresis and by fractional precipitation and chromatography. To avoid unfounded comparisons, analyses of samples of salmon sera were accompanied by analyses of samples of bovine serum. Results of analyses of sera or plasma of other animals performed on other occasions, with methods identical or similar to the methods employed in the present study, may be found in previous publications (Grant et al. 1966, 1967; Gaunce and Anastassiadis 1967).

Experimental Procedures

Blood from sockeye salmon (*Oncorhynchus nerka*), chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), cutthroat trout (*Salmo clarki*), and rainbow trout (*S. gairdneri*) was obtained by severing the caudal peduncle. Individual blood samples were allowed to clot at ambient temperature for 1–2 hr, stored on ice for 5–6 hr, and then centrifuged. The supernatant was centrifuged and the resultant sera samples stored frozen until analyzed. For the preparation of the bovine (domestic cattle *Bos typicus*, subspecies *primigenius*) serum, blood was allowed to clot at room temperature for 10–12 hr. The supernatant was decanted into tubes and centrifuged for 15–20 min at 3000 *g*. The serum was decanted and stored frozen.

The following hydrolysis procedures were used. For release of hexosamine, hexoses, and hexuronic acid, 1 ml of serum, or 10–20 mg of a protein fraction, were heated in sealed tubes with a suspension (4 ml) of sulphonated polystyrene resin (Dowex 50W-X8, 200–400 mesh) in 0.075 N HCl (1:2, w/v) for 36 hr at 100 C in a special oven equipped with a rotating vertical disc (Anastassiadis and Common 1958). For release of sialic acid, 2 mg of protein and 3 ml of 0.1 N sulphuric acid, or 0.1 ml of serum or plasma and 1 ml of 0.1 N sulphuric acid, were heated in sealed tubes in the special oven. The contents of the tubes were transferred to 5-ml volumetric flasks and made to volume. For release of fucose, 2–5 mg of protein, or 0.5–1.0 ml of serum or plasma, were heated, either with 2 ml 0.1 N sulphuric acid for 2 hr, or with 4 ml of the resin suspension for 12 hr, at 100 C in the special oven. Hexosamine was

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determined by the *p*-dimethylaminobenzaldehyde method (Anastassiadis et al. 1961), and hexose by the anthrone method (Fairbain 1953), after the hydrolysis products were eluted from the resin as previously described (Anastassiadis and Common 1958; Haab and Anastassiadis 1961). Sialic acid was determined by the thiobarbituric acid method (Warren 1959) (N-acetylneuraminic acid was used in preparing the calibration curve), fucose by the frozen anthrone method (Tsiganos and Muir 1966), and hexuronic acid by the carbozole method (Dische 1947). Protein was determined by the biuret method (Weichaelbaum 1946). Seromucoid was determined as the perchloric acid-soluble, phosphotungstic acid-insoluble fraction of the serum, essentially as reported by Winzler (1955) but with a difference in the amount of serum used (with proportional change in amount of reagents).

Some remarks on the analytical significance of the results for the glycoprotein constituents of the serum are necessary. For practical purposes, all the hexosamine, sialic acid, and fucose should be considered protein-bound. Only part of hexose is protein-bound, and free hexose varies substantially from time to time, even for the same animal. The apparent "hexuronic acid" content reported here should not be considered as a reliable value for this substance. There is no direct evidence that a substantial amount of hexuronic acid exists in the blood. The color that developed when the water eluate of the serum hydrolysate (and much more the serum itself) was treated with carbazole may be due to a great extent to other undetermined substances existing in the eluate.

Results and Discussion

In the summer and fall of 1964, sera of sockeye, chinook, and coho salmon, and cutthroat and rainbow trout were obtained from the Great Central lake system on Vancouver Island, B.C., and analyzed for hexosamine, sialic acid, fucose, and protein (Table 1). As serum hexosamine, sialic acid, and fucose are bound to protein, the individual values of the above constituents were expressed in relation to protein (ratio of serum content of a constituent × 100 to serum protein content), and average ratios and standard deviations of the ratios were calculated (Table 2). As the ratios of sialic acid to hexosamine and fucose to hexosamine characterize the carbohydrate part of serum glycoproteins, these two ratios were computed also (Table 2). There are indications of species, sex, and reproductive stage differences. However, differences in content of constituents among the reproductive stages were so large, and proportions of number of animals of the various reproductive stages in the sexes and species were so different, that a presentation of results of tests of statistical significance of differences among species and between sexes would be misleading. Furthermore, tests for differences between reproductive stages were justified, on the basis of adequate number of sampled animals, only between

TABLE 1. Hexosamine (base), sialic acid, fucose, and protein content of some fish sera. (Numbers in parentheses indicate number of sera (each from a different animal) analyzed when less than the whole group.)

	No.	Hexosamine (*mg/100 ml*)	Sialic acid (*mg/100 ml*)	Fucose (*mg/100 ml*)	Protein (*g/100 ml*)
Sockeye salmon	25	63.5±2.3(23)	65.7±6.3	3.3±0.3(16)	4.1±0.2
Males	14	66.0±2.9	71.6±9.8	3.2±0.5(8)	4.3±0.2
Sexually maturing (early)	5	66.8±6.0	101.9±19.6	2.7(3)	4.6±0.6
Almost sexually mature	1	61.0	38.0	6.2	4.2
Sexually mature	5	64.8±6.7	64.9±9.6	2.8±0.5(4)	3.8±0.5
Spawning	2	63.6	36.1		4.6
Finished spawning	1	77.7	58.0		4.7
Females	11	59.5±2.6(9)	58.3±7.1	3.4±0.3(8)	3.9±0.2
Sexually maturing (early)	1	–	103.4	–	4.2
Almost sexually mature	4	61.7±3.3	67.5±1.4	3.0±0.3	4.2±0.1
Spawning	3	62.4(2)	53.1	4.5(2)	4.0
Finished spawning	3	54.8	36.0	3.0(2)	3.4
Chinook salmon					
Sexually maturing males	4	99.0±7.1	74.6±16.5	5.8(1)	5.6(3)
Coho salmon	4	97.0±3.4	133.5±3.7	9.1(1)	5.6(2)
Sexually maturing males	2	101.0	130.5	9.1(1)	5.6(2)
Sexually maturing females	2	94.0	136.5		
Cutthroat trout	1	99.0	147.1		5.4
Sexually maturing male					
Rainbow trout	1	95.0	125.9		5.4
Sexually maturing male					

TABLE 2. Ratios of (hexosamine (H):protein (P)) X 100; (sialic acid (SA):P) × 100; (fucose (F):P) × 100; SA:H; and F:H in some fish sera. (Numbers in parentheses indicate number of sera (each from a different animal) analyzed when less than the whole group.)

	No.	(H:P)×100	(SA:P)×100	(F:P)×100	SA:H	F:H
Sockeye salmon	25	1.55±0.05(23)	1.57±0.12	0.08±0.01(16)	1.00±0.09(22)	0.030±0.004(15)
Males	14	1.56±0.07	1.63±0.17	0.08±0.01(8)	1.06±0.12(14)	0.048±0.007(8)
Sexually maturing (early)	5	1.48±0.10	2.12±0.28	0.06(3)	1.34±0.33	0.041(3)
Almost sexually mature	1	1.45	0.90	0.15(1)	0.62	0.101(1)
Sexually mature	5	1.73±0.13	1.71±0.06	0.08±0.01(4)	1.01	0.043±0.003(4)
Spawning	2	1.36	0.76		0.53	
Finished spawning	1	1.65	1.23		0.75	
Females	11	1.54±0.19(9)	1.50±0.17	0.09±0.01(8)	0.90±0.10(8)	0.051±0.001
Sexually maturing (early)	1		2.46			
Almost sexually mature	4	1.48±0.09	1.62±0.05	0.07±0.01	1.10±0.05	0.048±0.003
Spawning	3	1.45(2)	1.38	0.11(2)	0.92(2)	0.08(2)
Finished spawning	3	1.67	1.14	0.09(2)	0.67	0.05(2)
Chinook salmon						
Sexually maturing males	4	1.76(3)	1.20(3)	0.10(1)	0.79	0.059(1)
Coho salmon	4	1.73(2)	2.38(2)	0.16(1)	0.38	0.090(1)
Sexually maturing males	2	1.81	2.35	0.16(1)	1.30	0.090(1)
Sexually maturing females	2				1.46	
Cutthroat trout						
Sexually maturing male	1	1.83	2.76		1.49	
Rainbow trout						
Sexually maturing male	1	1.76	2.33		1.33	

sexually maturing (early) and mature male sockeye salmon. None of the differences in content of constituents and of their ratios between the two stages were found statistically significant at $P = 0.1$ for a two-tailed t-test. (The difference of sialic acid content was almost significant, as the calculated value of t was 1.844 and the tabulated two-tailed value of t for $P = 0.1$ and 8 df was 1.860.) For the following constituents and ratios, the differences between the two stages were found significant at $P < 0.1$ for a one-tailed t-test:sialic acid and protein content, and hexosamine-to-protein and sialic acid-to-protein ratios.

The analytical work on characteristic glycoprotein constituents in serum was concentrated on one species, the sockeye salmon, and on two distinct parts of its reproductive cycle. On July 28, 1967, serum samples from 19 sexually maturing sockeye salmon were obtained from fish a few days after they left the ocean on their spawning migration. The animals of this population are beginning to mature when they enter fresh water and ripen in the lake prior to spawning in October–November. The sera were analyzed for hexosamine, sialic acid, fucose, hexose, "hexuronic acid," seromucoid, and protein. On November 20, 1967, sera of 12 spawning sockeye salmon were obtained from fish on the spawning grounds of Cultus Lake, B.C., and analyzed. Because there were some differences in results, values and ratios obtained on the two occasions are given separately in Tables 3 and 4.

The average concentration values obtained in summer and fall suggested a slight alteration (usually a reduction) of apparent values of the glycoprotein constituents, as a result of storage of samples in the frozen state. However, results of t-tests for paired comparisons (Table 5) indicated that this possibility is remote.

There was a substantial difference in the protein content and in the content of the characteristic carbohydrate parts of the glycoproteins, between the serum of salmon and the serum of cow (see also results on composition of bovine serum in a previous publication (Grant et al. 1966)). The protein content of sockeye salmon serum was substantially lower than that of the cow. The ratios hexosamine to protein and sialic acid to protein indicated that salmon serum protein was much higher in hexosamine and sialic acid than in bovine serum protein. The ratio sialic acid to hexosamine was higher in salmon serum (and presumably in serum protein) than in bovine serum protein. As sialic acid occurs in terminal positions on branches of the serum glycoprotein chains, this result suggests that serum glycoproteins of the salmon are more branched than those of the cow. A statistical evaluation of species differences between sockeye salmon and cow has to be based on the following two assumptions: first, that sockeye salmon at two reproductive stages were a representative sample of the whole cycle of the species or of the adult part of the cycle; second, that values of constituents of bovine sera obtained on other occasions were essentially the same as values that would have been obtained if analyzed on the present occasion. It was felt that no statistical comparison of species differences should be given at the present time.

Comparisons of the means of sexes and of the two reproductive stages studied (Table 6) indicate that the most noticeable sex differences were for spawning salmon, in which hexosamine and sialic acid contents of serum of the female were significantly lower (at $P < 0.1$) than of the male. Furthermore, the ratio sialic acid to protein was significantly lower in the mature female than that in the mature male. The most noticeable reproductive stage differences were as follows: almost all constituents were substantially and significantly (at different probability levels) lower in mature than in maturing fish. The ratios of the constituents and the level of significance of the differences between maturing and mature indicated that the hexosamine and sialic acid content of serum glycoproteins for both sexes were higher in the mature than in the maturing fish. Although the present comparisons between maturing and mature salmon involve different populations (Great Central Lake fish and Cultus Lake fish), it is believed that they are valid. Similar differences in serum protein were reported by Jonas and MacLeod (1960).

Seromucoids from sera of six male and six female spawning sockeye salmon and from sera of three cows were precipitated and analyzed for hexosamine, sialic acid, and fucose (Table 7). It appeared that the fucose content of seromucoid of spawning salmon was lower than that of the cow. The only sex difference found statistically significant ($P < 0.025$) was the difference in the amount of sialic acid in the seromucoid.

A comparison of the values in Tables 3 and 7 indicates that only small fractions of the total amounts of hexosamine, sialic acid, and fucose in serum were precipitated in the seromucoid fraction — 5.4% of hexosamine, 4.8% of sialic acid, 29.1% of fucose, and 6.4% of hexose, for serum of spawning sockeye salmon. The total carbohydrate content of glycoproteins with large carbohydrate–protein ratio (which are those precipitated in the seromucoid fraction) represented only a small fraction of the total carbohydrate

TABLE 3. Hexosamine (base), sialic acid, fucose, "hexuronic acid," seromucoid, and protein content of sera of sockeye salmon. Composition of bovine sera is given for comparison. (Numbers in parentheses indicate number of sera (each from a different animal) analyzed when less than the whole group.)

Group		Hexosamine (*mg/100 ml*)	Sialic acid (*mg/100 ml*)	Fucose (*mg/100 ml*)	Hexose (*mg/100 ml*)	"Hexuronic acid" (*mg/100 ml*)	Seromucoid (*mg/100 ml*)	Protein (*g/100 ml*)
Sexually maturing (early)	19	87.5±3.2(18)	92.1±3.9(18)	16.6±0.8	136.1±12.9	10.7±0.6	214.5±1.0(12)	6.1±0.3(18)
Males	12	87.8±4.2	93.3±4.7(11)	15.7±1.1	157.4±16.2	10.1±0.8	216.6±0.6(8)	5.8±0.3
Summer	12	90.1±6.1	97.3±7.8(7)	17.0±2.3(8)	138.8±17.0(6)	9.5±1.3(8)	215.2±0.4(5)	5.8±0.4(11)
Fall	12	85.7±3.4(11)	94.4±4.3(11)	15.0±1.0(11)	155.0±18.0(11)	9.9±0.4(10)	217.8±0.7(5)	5.7±0.4
Females	7	86.8±5.2(6)	91.7±7.9	18.1±0.9	99.6±13.9	11.6±0.7	210.1±1.0(4)	6.7±0.4(6)
Summer	7	93.3±5.7(6)	87.8±7.7	21.1±2.6	84.7±16.4	10.9±1.5(6)	212.0(3)	6.6±0.5(6)
Fall	7	80.2±7.0(6)	89.9±7.2(6)	14.7±1.7(6)	160.0(2)	11.8±1.2(6)	208.3(3)	6.8±0.5(6)
Sexually mature (spawning)	12	58.8±1.8	66.8±5.1	3.3±0.2	94.8±4.7	2.5±0.3	206.0±8.7	3.7±0.1
Males	6	61.8±1.9	77.0±5.2	3.6±0.3	107.2±2.7	2.0±0.3	217.3±9.2	3.7±0.1
Females	6	55.8±2.7	56.5±6.6	3.1±0.4	82.5±5.2	3.1±0.3	193.7±13.9	3.7±0.1
Cow								
Present work	3	93.0	71.0	18.8	153.3	3.3	268.8	8.3
Previous work (Grant et al. 1966)	11	86.2±3.9	58.3±2.2	8.7±0.5				

TABLE 4. Ratios of (hexosamine (H):protein (P)) × 100; (sialic acid (SA):P) × 100; (fucose (F):P) × 100; (seromucoid (SM):P) × 100; SA:H; and F:H of sera of sockeye salmon. Corresponding ratios for bovine sera are given for comparison. (Numbers in parentheses indicate number of sera (each from a different animal) analyzed when less than the whole group.)

Group	No.	(H:P)×100	(SA:P)×100	(F:P)×100	(SM:P)×100	SA:H	F:H
Sexually maturing (early)	19	1.48±0.08(18)	1.56±0.10(17)	0.284±0.020(18)	3.59±0.26(11)	1.16±0.06(17)	0.194±0.010(18)
Males	12	1.56±0.11	1.63±0.13(11)	0.283±0.028	3.79±0.31(8)	1.18±0.07(11)	0.187±0.014
Summer	12	1.58±0.13(11)	1.41±0.18(9)	0.316±0.055(8)	3.85±0.39(5)	1.18±0.16(9)	0.199±0.036(8)
Fall	12	1.62±0.13(11)	1.75±0.14(11)	0.278±0.026	3.99±0.41(5)	1.11±0.05(10)	0.176±0.176(11)
Females	7	1.32±0.08(6)	1.45±0.16(6)	0.285±0.030(6)	3.01(3)	1.12±0.19(6)	0.208±0.020
Summer	7	1.43±0.10(6)	1.32±0.14(6)	0.351±0.067(6)	2.65(2)	1.03±0.10(6)	0.242±0.047
Fall	7	1.20±0.12(6)	1.52±0.21(6)	0.218±0.024(6)	3.05(3)	1.23±0.04(5)	0.173±0.020
Sexually mature (spawning)	12	1.61±0.06	1.82±0.14	0.090±0.007	5.85±0.20	1.13±0.09	0.056±0.002
Males	6	1.68±0.07	2.10±0.18	0.095±0.079	5.88±0.24	1.25±0.10	0.057±0.003
Females	6	1.54±0.09	1.54±0.17	0.085±0.012	5.82±0.34	1.02±0.13	0.054±0.004
Cow							
Present work	3	1.13	0.86	0.23	3.25	0.78	0.20
Previous work (Grant et al. 1966)	11	1.05±0.04	0.73±0.03	0.10			

TABLE 5. Values of t and df for differences in values of constituents found in July and November samples of sockeye salmon.

	Protein	Hexosamine	Sialic acid	Fucose	Hexose	"Hexuronic acid"	Seromucoid
Males							
t	0.94	0.97	0.76	0.72	2.40	1.27	–
df	10	10	6	6	4	5	
Females							
t	0.91	1.78	2.01	1.58	–	–0.09	–
df	5	5	5	5		4	
Males and females							
t	0.56	1.86	0.06	1.71	–	0.49	0.01
df	16	16	12	12		10	3

content of serum proteins. The higher proportion of fucose precipitated in the seromucoid fraction than that of hexosamine and sialic acid may indicate that a larger proportion of fucose than of hexosamine and sialic acid exists in glycoproteins of high carbohydrate content.

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(TABLES 6 and 7 *opposite*)

TABLE 6. Values of t and df for differences in values of serum constituents and of their ratios between sexes and two reproductive stages of sockeye salmon.

		Values of t						
	df	Hexosamine	Sialic acid	Fucose	Hexose	Hexuronic acid	Seromucoid	Protein
Concentration values								
Sexually maturing: males vs. females	17	0.15(16)[a]	0.42	1.49	2.67* [b]	–0.45		–1.52(16)
Sexually mature: males vs. females	10	1.84*	2.44*	1.09	4.16**	–2.38*	1.36	0.37
Males: maturing vs. mature	16	4.18**	2.18(15)*	7.63**	2.13*	6.94**	–0.09(12)	4.18**
Females: maturing vs. mature	11	5.29(10)**	3.37**	14.77**	3.50**	1.68	0.89(8)	6.88(10)**
Both sexes: maturing vs. mature	29	6.78(28)**	4.00(28)**	12.93**	2.46*	10.62**	0.97(22)	6.85(28)**
		(H/P)×100	(SA/P)×100	(F/P)×100		(SM/P)×100	SA/H	F/H
Ratios								
Sexually maturing: males vs. females	16	1.52(15)	0.83	–0.04		–	0.52(15)	–0.24
Sexually mature: males vs. females	10	1.26	2.24*	0.73		0.13	1.40	0.64
Males: maturing vs. mature	16	–0.73(15)	–2.13	4.52		–5.07(12)	–0.50(11)	1.60
Females: maturing vs. mature	10	–1.85*	–0.39	6.03**		–	0.68	3.67**
Both sexes: maturing vs. mature	28	1.18	–2.02(27)**	7.25**		7.03(21)**	0.28(27)	10.75**

[a] A number in parentheses indicates the number of df if this number is less than the one indicated in the df column.
[b] *Significant at a probability level $0.1 > P > 0.01$ (two-tailed test). **Significant at a probability level $P < 0.01$ (two-tailed test).

TABLE 7. Precipitated seromucoids, and hexosamine (H), sialic acid (SA), fucose (F), hexose (He), and hexuronic acid found in precipitated seromucoids (in mg/100 ml serum), and ratios of SA/H, F/H, and He/H.

Group		Seromucoid	Hexosamine	Sialic acid	Fucose	Hexose	Hexuronic acid	SA/H	F/H	He/H
Salmon	12	214.3±6.1	3.13±0.18	3.19±0.26	0.96±0.05	6.06±0.30	1.48±0.11	0.41±0.08	0.22±0.05	1.88±0.20
Male	6	217.3±9.2	3.05±0.23	2.58±0.36	1.02±0.10	5.70±0.13	1.52±0.15	0.53±0.15	0.23±0.08	1.70±0.36
Female	6	211.3±8.7	3.20±0.30	3.72±0.20	0.90±0.04	6.42±0.56	1.43±0.17	0.29±0.02	0.21±0.06	2.06±0.19
Cow	3	268.8	3·67	3.86	2.36	7.50	1.27	1.09	0.65	2.04

Growth and Fecundity of Rainbow Trout (*Salmo gairdneri*) Exposed to Single Sublethal Doses of X-rays During the Eyed Embryo Stage

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When rainbow trout (*Salmo gairdneri*) were reared for 2 years after irradiation with 21, 83, and 203 roentgens (R) during the eyed stage, the irradiation had no demonstrable effect on survival, growth, or fecundity. The trout were reared at temperatures of 15.4 and 17.2 C in parallel experiments for 160 days after fertilization. At the end of 2 years, 65 female trout (and 56 males), averaging more than 1400 g in weight, produced 186,400 eggs. Egg production was not related to irradiation dose or temperature; however, there was a slight dose-related increase in number surviving to the eyed stage in most of the irradiated groups.

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THE experiments described in the following report are part of a series of studies designed to evaluate the effects of ionizing radiation on salmonoid fishes. The objective of this study was to determine, in rainbow trout (*Salmo gairdneri*), the long-term effects on survival, growth, and fecundity of single, sublethal doses of X-rays during the eyed-embryo stage.

Previous studies of X-irradiated salmonids have indicated that doses of 200 R or less retarded the development of the gonads (Welander et al. 1948; Foster et al. 1949; Kobayashi and Mogami 1958; and Harada et al. 1960). However, long-term effects of irradiation were not investigated, the damage being assessed on the immature trout.

Materials and Methods

The trout used in this experiment were the offspring of a cross (Donaldson 1970) between a rainbow and a steelhead trout. The eggs from this cross were fertilized March 19, 1968, divided into two groups, and incubated in Williamson troughs to the eyed stage (400 temperature units) at two different temperatures. Each group was then divided into four lots, which were X-irradiated with 0, 21, 83, and 203 roentgens (R) (Table 1).

Irradiation procedures consisted of administering single X-ray doses to a monolayer of approximately 60 eggs in a 3-inch diam plastic petri dish. The top of the eggs protruded slightly above the water in the dish. A Picker, deep therapy, X-ray machine, operating at 200 kv peak and 20 ma, was used to deliver 124 R per minute to the eggs. The tungsten target was 38 cm from the eggs and filters consisted of 1 mm of aluminum and 0.25 mm of copper. The half-value layer of the beam was 0.80 mm of copper.

Dose rate was measured by placing thermoluminescent lithium fluoride crystals in plastic bags in the midst of a group of eggs during irradiation. The dosimeters were calibrated with a Victoreen condenser R-chamber under simulated beam and backscatter conditions. Details concerning the range of doses, the number of eggs irradiated, temperature, and mortalities are given in Table 1.

The lower of the rearing temperatures was the ambient temperature of the water pumped from Lake Washington. While one group of four lots was held at ambient temperature, the other group was held at a temperature on the average 1.8 degrees C above ambient for a period of 160 days after fertilization of the eggs. The fish, about 10 cm fork length, were then cold-branded, to identify each of the eight lots, which were then placed together in an outdoor raceway.

Table 1 includes data on losses of fish due to accidental death, poaching, illegible brands — losses that were obviously due to causes other than irradiation. The trout were anesthetized with tricaine methanesulfonate during the branding procedures, to reduce injury to the fish. Fork lengths were measured to the nearest centimeter at 4–5 month intervals; weights were measured to the nearest gram.

Results

MORTALITIES

There was no consistent dose effect at either temperature. Mortalities appear to be slightly

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TABLE 1. Mortalities from March 19 to October 1, 1969, in rainbow trout irradiated at three dosages (in roentgens) in the eyed-egg stage, reared at two temperatures to October 1, 1968, then kept at the same temperature to October 1, 1969.

	17.2 C (12.3–20.4)				15.4 C (10.8–18.4)			
	0 R	21	83	203	0 R	21	83	203
Original no. eggs	60	52	48	39	111	51	52	56
Losses from factors other than irradiation by Oct. 1, 1968[a]	1	0	3	1	1	13	1	0
% mortality (excluding losses) by Oct. 1, 1968	10.2	30.8	17.8	26.3	7.3	15.8	9.8	16.1
Total losses from factors other than irradiation by Oct. 1, 1969[a]	35	21	4	10	18	17	22	19
Total mortality (excluding losses) by Oct. 1, 1969 (%)	32.0	58.1	31.8	41.4	21.5	17.6	36.7	32.4

[a]Losses due to accidental deaths, illegible brands, and poaching from outside raceways.

higher in fish reared in the warmer water when compared by similar dose groups, but the mean values are not significantly different as determined by chi-square. Mortalities calculated from the data obtained during the period ending October 1, 1968, showed a significant difference only between the 21 R and the 0 R groups, with mortalities of 30.8 and 10.2%, respectively ($P = 0.02$). Losses due to accidental death or poaching were considerable during the period from October 1, 1968, to October 1, 1969. Mortalities at the end of this period were not statistically different due to temperature or dose; however, sample sizes were inadequate to distinguish small mortality differences, and extraneous mortality factors could have masked effects.

GROWTH

An analysis of variance of the length and weight[1] measurements at each age (Table 2) showed no significant difference ($P > 0.01$) attributable to radiation regardless of rearing temperature. Although fry reared at 17.2 C were larger than fish reared at 15.4 C on January 13, 1969 ($P < 0.05$), there were no significant differences ($P > 0.01$) in subsequent measurements after the fish were placed together in water of the same temperature.

FECUNDITY

The first trout spawned on March 27, 1970, and 50% of the females had spawned by May 1, 1970, more than 2 months later than the spawning period of similar rainbows reared at the same hatchery. Both irradiated and nonirradiated fish were late in spawning, but the late spawning may have contributed to the high mortality of the eggs that developed in temperatures higher than normal for embryos of rainbow trout.

In all, 65 female trout were spawned with 56 males to produce 186,400 eggs, 99,900 of which survived to the eyed stage (Table 3). An analysis of variance revealed no significant differences in the number of eggs produced as a result of irradiation, temperature, or irradiation and temperature interaction ($P > 0.05$). Nevertheless, in most instances, the proportion of eggs surviving to the eyed stage was slightly greater in the irradiated groups than in the controls. In those groups with the same dose of irradiation, the number of eyed embryos was slightly greater in eggs of parents reared at the higher temperature. However, the number of eggs surviving to the eyed stage was tested for dose effects, temperature effects, and dose–temperature interaction, and no statistically significant differences were found ($P > 0.05$).

Discussion

This experiment has revealed that fecundity of rainbow trout is not affected by X-ray doses up to 200 R applied during the eyed-egg stage. Several studies have suggested that X-ray doses as low as 50 R may affect fecundity. Foster et al. (1949) found that significant mortalities were produced in eggs of rainbow trout from parents subjected to 50 and 100 R 3 months before spawning. The growth in weight and length of the ovary of rainbow trout fry was definitely impeded by X-irradiation doses as low as 100 R (Kobayashi and Mogami 1958).

[1]The statistical analysis was performed on the logarithms of the weights.

TABLE 2. Average total lengths (cm) and weights (g) on four occasions after irradiation at three dosages on March 19, 1968, the fish having been reared at two temperatures until October 1, 1968, and then kept in hatchery raceways.

Date	Dose (*roentgens*)	Rearing temp 17.2 C			Rearing temp 15.4 C		
		Length	Wt	No.	Length	Wt	No.
Aug. 15, 1968	0	10.9±1.15[a]	–	53	9.3±1.24	–	106
	21	10.7±1.38	–	38	9.8±1.32	–	35
	83	10.6±1.24	–	47	9.6±1.16	–	47
	203	10.7±1.57	–	31	9.1±1.37	–	39
Jan. 13, 1969	0	25.3±2.57	242.5± 73.10	27	25.0±2.99	235.8± 84.45	83
	21	27.3±3.19	315.3±114.57	22	24.0±3.24	208.2± 89.45	34
	83	26.3±3.01	280.0± 98.05	39	25.0±2.80	226.8± 69.37	37
	203	25.5±4.19	266.7±135.33	28	24.5±2.85	216.8± 77.90	39
June 11, 1969	0	38.0±3.32	770.9±196.00	25	38.2±3.60	790.8±226.98	82
	21	38.4±4.35	799.9±278.03	22	37.2±3.33	726.3±211.61	31
	83	38.7±3.19	805.9±195.43	38	37.8±4.36	751.2±250.54	36
	203	39.3±3.83	854.4±289.80	25	38.4±3.81	814.4±222.05	39
Oct. 16, 1969	0	45.9±2.90	1392.1±297.90	17	45.7±3.31	1387.6±345.22	73
	21	45.9±3.70	1364.8±318.43	13	45.8±3.11	1339.2±280.47	28
	83	45.9±3.27	1390.6±250.13	30	45.0±3.29	1288.8±302.41	19
	203	46.8±3.30	1423.7±348.48	17	46.1±3.38	1353.6±320.96	25

[a]One standard deviation of the mean.

TABLE 3. Comparison of the number of eggs obtained from rainbow trout parents irradiated at three dosages during the eyed-egg stage, reared at two temperatures to October 1, 1968, then kept at the same temperature to October 1, 1969.

Temp (C)	Dose (*roentgens*)	No. females	Eggs produced		Eggs surviving to eyed stage		
			Range	Avg	Range	Avg	%
17.2	0	7	1216–5074	2973	49–3343	1508	50.7
	21	2	2152–5345	3749	272–3926	2099	56.0
	83	8	1510–5918	3324	160–4000	2064	62.1
	203	8	1382–6191	2794	276–4470	1842	66.0
15.4	0	11	1397–5584	3062	2–3845	1315	42.9
	21	6	1044–4429	2401	601–2437	1285	53.5
	83	11	1233–6264	2728	127–2697	1213	44.5
	203	12	373–4106	2587	184–3649	1534	59.3

However, no investigator had made a long-term study to determine the fecundity and survival of the eggs from irradiated gonads. The differences in results indicated here may lie in the rather high regenerative capability of the gonads. The regenerative capabilities of salmonoid gonads has been indicated by Kobayashi and Mogami (1958) after doses of 100 R, although the gonads did not recover after 500 R.

It is also possible that there is strain variation in radiosensitivity. Differences in radiosensitivity in hybrid mice has been indicated by Rugh and Wolff (1958), and Ehling (1964) found that comparisons of radiation response must take into consideration a possible three- to fourfold difference between strains within species.

Other investigators have found considerable variation in the radiosensitivity of the germ cells related to the stage of development. Oakberg (1963) reported that evaluations of relative damage to the ovary of mammals must include the possibility that different cell stages are being compared. Egami and his co-workers (1967) came to similar conclusions with the fish *Oryzias latipes*.

Although there were no statistically significant differences in the number of eggs produced, there appeared to be a slight increase in the number of viable eggs, correlated with dose in five of the six irradiated groups. Stimulation by ionizing radiation was probably first recorded by Gilman and Baetjer (1904) on the initial acceleration of development of the newt and the chick. Increased productivity has been noted by Ayala (1966) in *Drosophila*, by Hahn and Ward (1967) in mice, and by Crenshaw (1965) in beetles, after irradiation. Increased productivity in fish was reported by Blaylock (1969) in *Gambusia affinis affinis*, which had been exposed to radioactive wastes for several generations.

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Bacterial Counts of Commercial Fish Diets

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TRUST, T. J. 1971. Bacterial counts of commercial fish diets. J. Fish. Res. Bd. Canada 28: 1185–1189.

Two diets formulated and offered for commercial fish production were examined to determine their microbial burden. The total count of aerobic bacteria ranged from 10^2 to 10^7 bacteria per gram of diet. The diets contained psychrophilic and thermophilic species and up to 10^4 aerobic and anaerobic spore-forming bacteria per gram. Enterococci and members of the Enterobacteriaceae, including species of *Salmonella*, were also present. Fluorescent pseudomonads were detected in 18 of the 47 samples tested. Proteolytic, amylolytic, lipolytic, and haemolytic organisms were shown to be present in measurable numbers. The total viable aerobic count did not change markedly on storage of representative samples at 4, 20, or 30 C.

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THE presence of bacteria in human and animal feeds is well established. That these foods or feeds can serve to distribute bacteria pathogenic to man and animals is equally well documented (Bowmer 1964; Thatcher and Clark 1968). This has led to the establishment of tolerance limits for pertinent categories of microorganisms in specific foods (Powers et al. 1970; Statutory Orders and Regulations 1955). Janssen (1970) has reviewed the extensive literature that suggests that fish may be more important vectors of human disease than has generally been realized. Since there is relatively little information available concerning the microflora present in fish diets, studies have been initiated to ascertain if fish feeds, like animal and human foodstuffs, should have tolerance limits applied to them. This communication reports the presence of a wide variety of viable bacteria, including members of the genus *Salmonella*, in commercial fish feeds, and suggests that greater attention must be given to this microbial flora.

Materials and Methods

FISH DIETS

Through the courtesy of two producing fish hatcheries, samples from 47 50-lb bags of two commercially produced fish diets were obtained between November 1969 and August 1970. These 47 samples represented nine separate production batches. Standard bacteriological sampling techniques were followed in the procurement of the samples (American Public Health Association 1958). The samples were held at 4 C until submitted to bacteriological examination.

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BACTERIOLOGICAL EXAMINATION

(*a*) *Viable counts*

Using the procedure outlined in the American Public Health Association (1965), a 10-g sample was transferred aseptically to a dilution bottle containing 90 ml of buffered-distilled water, shaken vigorously 25 times, and duplicate serial dilutions were prepared in buffered-distilled water. The viable bacteria present in six bags of diet A and two bags of diet B, chosen at random, were enumerated by the Surface Plate Method recommended by the International Standards Organization (Thatcher and Clark 1968). Nutrient agar (Fisher) incubated at 5, 30, 37, 44, and 65 C, Trypticase Soy agar (Baltimore Biological Laboratories) at 30 C, and Tryptose Glucose Yeast Extract agar (Fisher) at 30 C were used to estimate viable aerobic bacteria. The total viable anaerobic count was also determined on nutrient agar at 30 C, as were the aerobic and anaerobic spores after treatment of the 10^{-1} dilution at 100 C for 5 min. Clostrisel agar (BBL) and Trypticase Soy Neomycin agar (BBL) incubated anaerobically at 30 and 37 C were used to detect the presence of clostridia. Anaerobiosis was obtained using BBL Anaerobic Jars with Disposable Hydrogen–Carbon Dioxide Generator envelopes (BBL). MacConkey agar (BBL), *Salmonella–Shigella* agar (Fisher), Bismuth Sulfite agar (Difco), and Urease agar (BBL) were incubated aerobically at 37 C to enumerate members of the Enterobacteriaceae. Fecal streptococci were enumerated on M-*Enterococcus* agar (BBL) incubated at 37 C. The number of proteolytic organisms present was determined by the production of casease on skim milk agar plates (Salle 1967); the lipolytic organisms were determined by detecting the production of lipase on nutrient agar plates containing 1% (w/v) tributyrin; and the amylolytic organisms present were determined by the production of amylase on nutrient agar plates containing 0.2% (w/v) soluble starch. Haemolytic organisms were determined on blood agar

at 37 C. An agar containing 3% (w/v) fish diet (A or B) was used to detect bacteria able to grow using the diet as sole source of nutrients. Malt agar (Fisher) and Sabouraud Dextrose agar (BBL) were employed to detect molds. Samples of both feeds were examined for fluorescent pseudomonads by plating on King's B agar (King et al. 1954), and for pigment-producing species of *Aeromonas* using Furunculosis agar (Difco) incubated at 30 C.

(b) Most probable number (MPN) estimates

The procedures followed were those of the American Public Health Association (1965). The eight bags chosen for the viable counts were also examined for coliforms by the 10:1:0.1 multitube method using MacConkey broth (BBL) and Brillant Green Bile broth (BBL). Fecal coliforms were confirmed by separate loop transfers from the positive tubes to MacConkey broth and incubation at 44.5 ± 0.5 C.

(c) Enrichments

All 47 samples were examined for fluorescent pseudomonads and pigment-producing aeromonads after enrichment of 1 g of diet in 9 ml of King's B broth or Furunculosis broth. Similarly, Selenite-F broth (BBL) and Tetrathionate broth (BBL) enrichments at 37 C followed by plating on *Salmonella–Shigella* agar, Brilliant Green agar (BBL), and Deoxycholate Citrate agar (BBL), were employed to detect *Salmonella*. Incubations at 65, 44, and 37 C were for 24 hr, at 30 C were for 48 hr, and at 5 C were for 10 days.

(d) Additional tests

The additional tests used to partially characterize the isolates were those described by Skerman (1967).

Results

The results in Table 1 show that the dry fish feeds examined carried significant numbers of bacteria. Mesophilic bacteria predominated (10^3–10^7/g), but psychrophilic species ($<10^3$–10^6/g) and thermophilic species (10^3–10^5/g) were also present. Many of the mesophilic species (10^3–10^6/g) were able to grow on the media, which contained 3% (w/v) fish diets A or B. Aerobic and anaerobic spore-forming bacteria ($\leqslant 10^3$–10^4/g) were present in the feeds, but the anaerobic spore-forming organisms were unable to grow on Clostrisel agar or Trypticase Soy Neomycin agar, which are selective for clostridial species pathogenic to man.

The fish diets contained lactose-fermenting and nonlactose-fermenting enteric bacteria ($<10^3$–10^5/g). Although direct plating of the samples on *Salmonella–Shigella* (or Bismuth Sulfite agar) failed to isolate *Salmonella* species, the use of a preliminary enrichment in Selenite-F broth or Tetrathionate broth, followed by plating on media selective for *Salmonella*, did result in the isolation of *Salmonella* from 5 of the 35 samples of diet A examined. Slide agglutination using *Salmonella* "O" and "H" antisera (BBL) identified the isolates as *S. montevideo*, *S. livingston*, and *S. anatum*. No *Salmonella* were isolated from diet B. The count of fecal streptococci ranged between 10^2 and 10^3/g.

The diets also contained 10^3–10^4 organisms/g capable of producing either casease, amylase, lipase, or haemolysin. Fluorescent pseudomonads were found in 25 samples of diet A and three samples of diet B. Sample 1 of diet A contained 4×10^5 fluorescent pseudomonads/g of ration, and sample 1 of diet B contained 4×10^3/g. Only one sample of diet A contained bacteria able to produce a brown water-soluble pigment during growth on Furunculosis agar. This gram-negative, oxidase-positive, motile rod was able to ferment glucose and was subsequently identified as *Aeromonas* sp. The diets contained fewer than 200 molds/g.

The number of coliforms present in eight samples of diets A and B was estimated by the MPN multitube procedure and ranged between 140 and 1750 (mean 500)/100 g of dry feed. The number of fecal coliforms able to ferment lactose at 44 ± 0.5 C ranged between 20 and 790 (mean 174)/100 g.

The agar containing 3% (w/v) fish diet (A or B) supported growth of *Aeromonas liquefaciens* American Type Culture Collection (ATCC) 14715, *A. hydrophila* ATCC 9071, *A. salmonicida* ATCC 14174, and *Mycobacterium marinum* ATCC 927. Apart from these fish pathogens, the three species of *Salmonella* isolated from the diet samples also grew well on the media containing diet A or diet B.

The total viable aerobic count did not increase on storage. Diet A was stored at 4, 20, and 30 C for up to 72 days, and Nutrient agar plates were inoculated and incubated at 30 C for 48 hr. The estimated number of organisms ($\times 10^2$) per gram of diet were as shown:

Temp of storage (C)	Days of storage	No. organisms ($\times 10^2$)/g of diet A
4	0	1200
	7	1000
	28	1000
	56	720
	72	270
20	0	1200
	7	1700
	28	1500
	56	410
	72	200
30	0	790
	7	620
	28	500
	56	500
	72	600

TABLE 1. Estimated microbial burden in commercial dry fish diets (produced and tested in 1970 except as noted).

	No. organisms × 10^3/g of diet							
	Diet A						Diet B	
Sample:	1	2	3	4	5	6	1	2
Date produced/tested:	Jan./Apr.	Jan./Apr.	Feb./July	Feb./July	Feb./Aug.	Feb./Aug.	Nov. 69/Aug.	Nov. 69/Aug.
Aerobic incubation (*C*)								
Nutrient agar, 5	1200	50	50	210	220	22	<1	32
Nutrient agar, 30	120000	5500	†90	1200	790	1100	50	680
Trypticase soy agar, 30	160000	5700	1900	1300	910	1400	150	570
Tryptose glucose yeast extract agar, 30	150000	5300	1500	1300	880	910	94	570
3% fish diet A agar, 30	90000	2900	450	220	380	650	86	780
3% fish diet B agar, 30	95000	2700	130	150	350	600	13	370
Nutrient agar, 37	9500	6700	1300	1500	980	1200	300	890
MacConkey agar, 37 (lactose +)	23	340	1	4	1	2	<1	2
Salmonella–Shigella agar, 37 (lactose –)	12	8	6	1	9	13	<1	13
M-*Enterococcus* agar, 37	10	4	4	7	20	7	3	7
Nutrient agar, 44	1600	3400	450	250	680	690	390	600
Nutrient agar, 65	2100	500	10	10	20	20	310	180
Aerobic spores								
Nutrient agar, 30	400	10	3	7	1	7	24	15
Anaerobic incubation								
Nutrient agar, 30	1900	220	890	220	90	90	28	240
Anaerobic spores								
Nutrient agar, 30	300	50	70	30	<1	<1	<1	<1
Enzyme production								
Skim milk agar, 30 (casease +)	200	450	10	90	400	800	890	380
Starch agar, 30 (amylase +)	300	9	11	30	360	330	350	120
Blood agar, 37 (haemolysin +)	100	330	380	330	480	330	750	180
Tributyrin agar, 30 (lipase +)	900	60	280	230	340	340	270	150

Discussion

Commercial foods and animal feeds are routinely examined for the presence of organisms pathogenic to man and animals. It is difficult, however, to determine specific pathogenic microorganisms when the pathogen is sparsely distributed in material heavily contaminated with other organisms. This has led to the widespread use of groups (or species) that are readily enumerated and whose presence in foods (or feeds), within numerical limits, is considered to indicate the possibility of excessive numbers of pathogenic or toxigenic species. Tests for these "indicator" organisms have proved valuable in assessing both the microbiological safety and quality of foods. For human foods, the choice of "indicator" organisms depend on the type of food; included are the aerobic mesophilic bacteria, enteric bacteria including *Escherichia coli* and other coliforms, the enterococci and *Salmonella*, *Streptococcus salivarius*, *Staphylococcus*, and clostridia. For animal feeds and poultry feed, *Salmonella* has been an important indicator (Thatcher and Clark 1968).

For fish diets, however, different indicators are recommended. In this study, psychrophilic and mesophilic aerobes were enumerated because the storage life of the feed could be affected by these organisms and because fish are held in water between 5 and 18 C. Also bacteria important to the public health are mesophilic. Bacteria able to produce extracellular enzymes could affect the quality of a ration or play a role in the infection of fish or man and were included as "indicator" organisms, as were the thermophiles and spore-forming bacteria that resist pasteurization. Of the species commonly pathogenic to fish (Bullock 1961, 1964) the fluorescent pseudomonads and the pigment-producing aeromonads are the easiest to detect. Coliforms, fecal coliforms, enterococci, and organisms able to grow on *Salmonella–Shigella* agar are indicators of fecal contamination and of bacteria potentially pathogenic for man. Selenite enrichment of the rations was included, since *Salmonella* infections of man and animals have been traced to foods, feeds, and feed ingredients such as fish meal (Bowmer 1964). Similarly, fish and fish products have been implicated in clostridial intoxication of man (Frazier 1967).

Foods and feeds are generally considered unsatisfactory when they contain a large population of bacteria, even if the bacteria are not pathogenic or have not altered the material. Decomposition of the material generally becomes evident in foods containing 10^8–10^9 organisms/g (Thatcher and Clark 1968). The viable aerobic count in one sample of diet examined in this study was in this range. Although no increase in viable bacteria was obtained in storage of these dry feeds, the ability of the bacterial flora to produce extracellular proteolytic, lipolytic, and amylolytic enzymes would favour spoilage if any increase in the moisture content of the diet occurred during storage. Commercial wet diets already have an increased moisture content that will favor the growth of the bacteria present and the production of extracellular enzymes.

The nutrients required for the growth of a wide range of bacterial species have been shown to be present in fish feeds (Table 1). It is therefore probable that uneaten feed in the water will provide nutrients for bacteria. The feed also contains a bacterial inoculum, either from the uneaten feed or after passage through the fish in the feces. Under hatchery conditions in tanks containing 40 fish of 20 g held in 100 liters of water at 13 C, a dry ration containing 10^6 organisms/g being fed at a rate of 1% fish body weight/day (Brett et al. 1969) will contribute an inoculum of 8×10^6 viable bacteria per day to the tank. Since bacteria are attracted to surfaces and become adsorbed thereto (Brock 1966), many of these bacteria may not be washed out of the tanks; heavy bacterial growth is thus likely to occur on tank surfaces in fish hatcheries. In crowded hatcheries, the aqueous environment may approach critical conditions arising from the fecal and other excretory products of the fish, and a lowered oxygen tension brought about by a high density of fish. Any bacterial growth will further lower the oxygen tension and increase the ammonia level of the water and perhaps lower the resistance of the fish. The presence in the ration of bacteria that are potentially pathogenic to fish further aggravates the situation. The presence of proteolytic and haemolytic bacteria in all batches examined, and of fluorescent pseudomonads and an aeromonad in other batches suggests that the microbial flora of the fish feed may cause some of the problems of fish mortality under crowded hatchery conditions.

The relatively high levels of indicator organisms found in the fish diets require consideration. The bacteriological limits for commercial gelatin are fewer than 10,000 aerobes/g and no coliforms per 0.01 g, whereas bone meal should contain fewer than 1000 aerobes/g and no *Escherichia coli* in 1 g (Statutory Orders and Regulations 1955). Any count of fecal coliforms is considered unacceptable in the production of fisheries products processed under Canadian Government inspection (Hayward and MacCallum 1969). The bacteriological requirements for a dehydrated human food (Powers et al. 1970) are a total aerobic plate count of $\leqslant 10{,}000$/g, a total coliform count of $\leqslant 10$/g, fecal coliforms negative per g, fecal streptococci $\leqslant 20$/per g, and no

Salmonella in 10 g. Although these requirements may be too rigorous for fish feeds, the numbers of fecal streptococci and fecal coliforms and the numbers of bacteria able to grow on *Salmonella-Shigella* agar in this study are unacceptable if the fish fed on the rations are for human consumption. Salmonellosis is a public health problem of serious magnitude (Bowmer 1964) and the presence of *Salmonella* in fish rations may present a hazard to workers handling and feeding fish, as well as to human and animal populations downstream from the hatchery.

Consideration should be given to pelleting fish rations under conditions allowing pasteurization. This would drastically reduce the numbers of bacteria present. Workers should be more aware of the microbial burden of fish rations, and certain bacteriological standards might need to be imposed on the manufacturers. Consideration should be given to applying the more rigorous sanitary procedures developed by the dairy and poultry industries in the management of hatcheries.

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NOTES

Amino Acid and Fatty Acid Composition of Tissues of the Dungeness Crab (*Cancer magister*)

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ALLEN, W. V. 1971. Amino acid and fatty acid composition of tissues of the Dungeness crab (*Cancer magister*). J. Fish. Res. Bd. Canada 28: 1191–1195.

Tissues of the Dungeness crab (*Cancer magister* Dana) were analyzed for content of total lipid, protein, amino acids, fatty acids, and glucosamine. Ovaries and hepatopancreas were the major lipid storage organs. Gonads (ovaries and testes) and skeletal muscle contained substantially more protein than the other tissues. All tissues except the exoskeleton had balanced amino acid compositions. The exoskeletal protein was deficient in arginine and lysine. Glucosamine was a prominent constituent of the exoskeleton and of the gills and the gastric apparatus. Palmitic, palmitoleic, oleic, eicosapentaenoic, and docosahexaenoic acids were the major fatty acids found in all tissues.

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THE Dungeness crab (*Cancer magister* Dana) provides a major fishery along the west coast of the United States. Except for reports on proximate and mineral composition (Nelson and Thurston 1964; Farragut and Thompson 1966), little information is available on the biochemical composition of *C. magister* tissues. This report provides data on the total lipid, protein, amino acid, fatty acid, and glucosamine content of six tissues: skeletal muscle, hepatopancreas, exoskeleton, gonads, hemolymph, and viscera.

Materials and methods — Crabs were provided by the California Department of Fish and Game. The animals were collected by trawl off the entrance to Humboldt Bay, California, in July 1969. Four crabs of each sex were selected for analysis. Hemolymph was collected from the animals. They were next heated at 50 C in sea water for 20 min to harden the tissues prior to dissection. This heat treatment is not comparable to that used for industrial processing of crabs. The biochemical data obtained apply, therefore, to raw rather than cooked crab tissues. Each animal was dissected into the tissue components: hepatopancreas, gonads, exoskeleton, and skeletal muscle. The remaining solid tissues (viz., heart, gills, gastric apparatus, and excretory organs) were pooled under the term "viscera." A body component index (wet weight of tissue/wet weight of intact animal, Giese 1959) was calculated for each tissue. The difference between the wet weight of an intact animal and the summed weights of its solid tissues was used to calculate the hemolymph weight and hemolymph index. Subsamples of the wet tissues of each animal were dried to constant weight in a vacuum desiccator. The dried tissues were used for the total lipid, fatty acid, amino acid, and glucosamine determinations.

Tissue proteins were analyzed by the method of Lowry et al. (1951) using bovine serum albumin as the standard. Total lipids were determined gravimetrically after extraction of the lipids from dried tissues with chloroform–methanol (Folch et al. 1957). All operations with the total lipids and fatty acids were performed in a nitrogen atmosphere.

Fatty acids were determined by gas–liquid chromatography (GLC) of the methyl esters. Methyl esters were prepared from the pooled total lipid extracts of the four specimens of each sex by transesterification with boron triflouride–methanol–benzene reagent (Morrison and Smith 1964). The methyl esters were analyzed on stainless steel columns (2.7 mm bore × 2.5 m length) packed with 10% (w/w) EGSS-X on 90/100 mesh Anakrom ABS. Operating conditions were: column oven, 200 C isothermal; injector port 225 C; detector, flame-ionization at 230 C; carrier gas flow, nitrogen at 30 ml/min. A Varian Aerograph 1860-1 chromatograph was used for both the fatty acid and amino acid analyses. Methyl esters were identified by comparing retention times with a primary standard containing even-chain saturated fatty acids (National Institutes of Health mixture F) and a secondary standard containing the major long-chain monoenoic and polyenoic acids (viz., cod liver oil fatty acids, Ackman and Burgher 1965). The separation of the methyl esters into saturation classes by argentation thin-layer chromatography prior to GLC was employed as an aid in identifying fatty acids (Allen 1968). Fatty acids were quantified with a Disc Integrator.

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Amino acids were determined by the gas–liquid chromatographic technique of Gehrke et al. (1968) using a modification for histidine quantification (Roach et al. 1969) and the modified ethylene glycol adipate (EGA) column described by Roach and Gehrke (1969). For each tissue, equal weights of lipid-free, dry samples from the four individuals of each sex were pooled and ground to a powder. Ten-milligram portions of the powdered tissue were hydrolyzed in vacuo at 110 C for 22 hr in 20 ml of redistilled 6 N HCl (Moore and Stein 1963). The hydrolyzates were analyzed directly by GLC for all amino acids except methionine, cystine, and tryptophan. Chloroform–methanol (2:1 v/v) was used to extract lipids from the tissues prior to hydrolysis of the tissue proteins. This solvent most probably also extracted most of the free amino acids of the tissues. Two of the principal unbound, nonprotein amino acids, ornithine and taurine, were not detected in the hydrolyzates. The amino acid compositions reported here apply, therefore, to the tissue proteins and do not include the contribution of the unbound, free amino acids, which occur in considerable proportions in crustacean tissues (Huggins and Munday 1968).

Tests revealed that cystine and methionine were completely destroyed by the hydrolytic conditions employed. Accordingly, separate subsamples of lipid-free, dry tissue were oxidized with performic acid prior to hydrolysis in order to convert cystine and methionine into the more stable cysteic acid and methionine sulfone, respectively (Blackburn 1968). The N-trifluoroacetyl (N-TFA), *n*-butyl ester derivative of methionine sulfone was analyzed on the OV-17 column described by Gehrke et al. (1968), appearing between the arginine and tryptophan peaks. The standard derivatization procedure given by Gehrke et al. (1968) for the preparation of N-TFA, *n*-butyl amino acids did not yield a cysteic acid derivative that could be chromatographed on either the EGA or OV-17 columns. Preliminary efforts to prepare a trimethylsilyl derivative of cysteic acid (by the method of Gehrke et al. 1969) that could be chromatographed were unsuccessful. Consequently, cysteic acid was separated as the dinitrophenyl derivative on thin layers of silica gel G (Pataki 1968) and quantified by reading the absorbtion at 360 nm.

Tryptophan was determined by the colorimetric procedure of Spies and Chambers (1949). Tissue samples were treated with 19 N H_2SO_4 in the presence of *p*-dimethylaminobenzaldehyde and then reacted with $NaNO_2$.

The chitinous tissues (exoskeleton and viscera) yielded glucosamine upon hydrolysis. The N-TFA derivative of glucosamine, which was produced by the procedure of Gehrke et al. (1968), was eluted on the EGA column between the tyrosine and lysine peaks. The glucosamine peak overlapped that of ornithine, the compound that is used as an internal standard for quantifying the amino acids. It was necessary, as a result, to use stearic acid as the internal standard when analyzing the amino acids of the exoskeleton and viscera.

Results and discussion — The animals used for these analyses were taken shortly after mating and molting of the females. The mean body component indices (wet weights of tissue/wet weights intact animals) for the various tissues of males and females were:

Tissue	Males	Females
Gonad	0.6±0.3[a]	3.8±2.1
Exoskeleton	33.2±2.4	32.1±3.4
Hepatopancreas	2.7±1.7	3.5±1.1
Viscera	4.6±0.8	4.2±0.9
Skeletal muscle	23.2±5.1	17.3±3.2
Hemolymph	35.7±7.2	39.2±7.5

[a]One standard deviation of the mean of four determinations.

The index for testes was very low, indicating a spent condition. The exoskeletons of the females were soft and the skeletal muscle index low. A decline in the indices of all tissues except hemolymph is expected following a molt as a consequence of the uptake of sea water during the molt. At this stage in the reproductive cycle the gonads and hepatopancreas comprised a small portion of the crabs. The major tissues were hemolymph, exoskeleton, and skeletal muscle.

For both males and females, more lipid was found in the gonads and hepatopancreas than in other tissues (Table 1). Predictably, more lipid was found in females than in males. The lipid content of skeletal muscle was about 1% in both sexes. The hemolymph lipids, which are important in the interorgan transport of fat, will be the subject of a subsequent report.

Gonads and skeletal muscle had higher protein contents than other tissues (Table 1). The exoskeletal protein content of the freshly molted, soft females (6.8%) was appreciably higher than for the hard males (4.4%). This finding agrees with that of Degens et al. (1967), who noted that the protein content of the exoskeleton of portunid crabs appeared to be inversely related to the degree of mineralization.

The proportions of amino acids found for skeletal muscle (Table 2) are in substantial agreement with the results reported for blue crab, *Callinectes sapidus*, by Konosu et al. (1958) and Thompson and Farragut (1966). Glutamic acid, aspartic acid, leucine, arginine, and lysine were the major amino acids in skeletal muscle. The methionine levels (reported as methionine sulfone) for all tissues were considerably higher than those given by Thompson and Farragut (1966) for muscle and offal of the blue crab. The exoskeleton contained a very low proportion of lysine and virtually no arginine. This result is not in accord with the data of Degens et al. (1967), who reported the presence of high proportions of lysine and arginine in the

exoskeleton of the blue crab. A meal derived in large part from exoskeletal waste of the king crab, *Paralithodes camtschatica*, also contains considerable lysine and arginine (Kifer and Bauersfeld 1969). The hemolymph amino acid composition agrees fairly well with an analysis of the amino acid composition of hemocyanin (the major hemolymph protein) of the blue crab (Ghiretti-Magaldi et al. 1966). Proteins other than hemocyanin are present in quantity in *C. magister* hemolymph (W. V. Allen unpublished data). Consequently, the amino acid composition of the total hemolymph proteins cannot be expected to coincide with that of hemocyanin. No sex differences were apparent in the amino acid composition of the tissues. As the gonads of the male specimens were spent, one would not expect to find in the testes the high proportions of arginine, lysine, and histidine often associated with protamines.

TABLE 1. Mean dry weights (as percents of wet weights except for hemolymph), total lipid, and protein of *C. magister* tissues.

Tissue	Dry wt	Total lipid	Protein
Females			
Ovary	36.6±7.9[a]	9.5±0.8	17.0 ±1.6
Exoskeleton	67.5±4.7	0.4±0.2	6.8 ±1.5
Hepatopancreas	28.0±7.3	11.9±2.8	7.1 ±0.5
Viscera	16.7±3.4	1.2±0.6	7.3 ±0.8
Skeletal muscle	21.8±1.9	1.1±0.2	13.8 ±0.6
Hemolymph	— —	— —	4.6[b]±0.8
Males			
Testis	24.8±4.0	3.0±0.5	15.2 ±2.0
Exoskeleton	68.3±7.3	0.3±0.1	4.4 ±0.3
Hepatopancreas	26.2±5.3	6.6±2.6	9.3 ±0.9
Viscera	17.7±3.6	1.2±0.2	6.7 ±1.1
Skeletal muscle	21.5±3.2	0.9±0.1	16.4 ±1.6
Hemolymph	–	–	5.0[b]±0.9

[a]One standard deviation of the mean of four determinations.
[b]Grams/100 ml of hemolymph.

The average glucosamine contents of the exoskeleton for males and females were 7.27 and 8.50%, respectively (Table 2). These values indicate an exoskeletal chitin content of 8.2% for males and 9.6% for females. Degens et al. (1967) reported similar values for the more mineralized regions of the exoskeletons of three portunid crabs. The viscera also contained considerable amounts of glucosamine (3.82% in females and 2.46% in males), which is derived from the chitin of the gills and gastric apparatus.

The fatty acid compositions of the tissues displayed the high proportions of long-chain, polyenoic acids usually found for marine lipids (Table 3).

TABLE 2. Amino acid compositions of tissues as grams amino acid/100 g tissue dry weight.

	Gonad		Exoskeleton		Hepatopancreas		Viscera		Muscle		Hemolymph[a]
Amino acid	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Male
Alanine	1.99	3.19	0.69	0.47	1.33	1.68	2.58	2.47	4.07	4.65	0.19
Arginine	3.74	3.33	Trace	Trace	2.42	2.04	2.96	1.96	7.02	7.25	0.20
Aspartic acid	4.29	6.36	1.14	0.65	3.12	4.15	4.87	4.29	6.19	7.00	0.94
Cysteic acid	–	0.61	–	0.03	–	0.17	–	0.28	–	0.90	0.04
Glutamic acid	5.78	6.37	1.03	0.75	3.40	4.77	5.55	4.45	8.44	9.89	0.53
Glycine	2.35	5.67	0.91	0.47	1.78	2.66	3.25	3.11	3.73	4.52	0.16
Histidine	1.74	2.18	0.11	0.15	1.05	1.35	2.57	1.47	2.64	3.39	0.18
Isoleucine	2.75	3.22	0.40	0.16	0.89	1.11	1.72	1.45	3.09	3.40	0.19
Leucine	3.79	4.57	0.68	0.31	1.50	2.15	2.96	2.54	5.09	6.26	0.35
Lysine	2.95	3.03	0.09	0.06	1.78	2.10	2.00	1.64	4.32	5.57	0.24
Methionine sulfone	–	4.75	–	0.39	–	2.30	–	1.36	–	4.39	0.11
Phenylalanine	2.19	2.64	0.68	0.36	1.13	1.49	2.20	1.87	2.77	3.03	0.33
Proline	2.39	3.79	0.57	0.31	0.97	1.08	2.39	2.40	2.51	2.76	0.18
Serine	2.99	3.44	0.92	0.47	1.58	1.96	2.29	2.02	3.16	3.54	0.24
Threonine	2.99	3.74	0.40	0.29	1.29	1.74	2.49	2.28	3.41	3.52	0.20
Tryptophan	1.10	0.81	0.11	0.08	0.49	0.54	0.67	0.72	1.16	0.91	0.11
Tyrosine	2.33	2.62	1.48	1.21	1.26	2.04	2.49	1.61	2.26	2.52	0.25
Valine	3.14	3.85	0.79	0.42	1.21	1.65	2.39	2.31	3.29	3.54	0.29
Glucosamine	0.00	0.00	8.50	7.27	0.00	0.00	3.82	2.46	0.00	0.00	0.00

[a]Grams/100 ml hemolymph.

TABLE 3. Fatty acid compositions of tissues as grams fatty acid/100 g total fatty acids.

Fatty acid[a]	Double-bond position[b]	Gonad		Exoskeleton		Hepatopancreas		Viscera		Muscle	
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
12:0	–	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
14:0	–	1.1	0.5	0.1	0.4	2.0	1.6	0.6	0.5	0.1	0.1
15:0	–	0.2	0.1	0.1	0.2	0.0	0.3	0.1	0.2	0.2	0.2
16:0	–	18.1	11.7	16.3	14.5	15.6	13.9	15.0	15.0	13.6	14.4
16:1	9	24.0	6.1	17.7	6.8	21.3	7.6	16.1	7.9	8.0	5.6
17:0	–	0.2	0.3	0.3	0.4	0.0	0.3	0.2	0.4	0.2	1.2
18:0	–	2.7	7.4	5.9	8.5	4.2	9.7	5.7	8.6	6.0	7.0
18:1	9	17.1	16.4	21.4	17.5	16.8	13.6	20.7	18.5	15.4	14.0
19:0	–	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
19:1	?	0.2	0.2	0.2	0.4	0.3	0.4	0.3	0.0	0.0	0.0
20:0	–	0.0	0.1	0.2	0.9	0.0	0.0	0.1	0.2	0.2	0.2
20:1	11	3.1	4.6	4.7	5.0	10.0	8.0	4.8	5.1	2.0	1.6
20:2	8,11	0.5	0.4	0.4	1.4	0.7	0.7	0.3	0.4	0.3	0.1
20:2	11,14	0.2	1.0	0.5	1.3	0.7	1.0	0.8	0.7	0.8	1.1
20:3	8,11,14	0.0	0.0	0.4	0.5	0.0	0.1	0.0	0.4	0.0	0.0
20:3	11,14,17	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:4	8,11,14,17	0.2	0.5	0.1	0.6	1.0	1.0	0.0	0:0	0.0	0.0
20:5	5,8,11,14,17	15.4	26.6	16.8	22.6	12.3	19.7	20.2	22.3	34.2	34.8
22:1	13	1.7	5.0	3.3	4.0	1.7	4.0	3.9	5.1	2.7	3.5
22:4	7,10,13,16	0.7	3.0	1.0	0.9	1.1	1.2	0.9	0.7	0.7	1.0
22:5	7,10,13,16,19	1.9	1.8	1.5	1.6	1.9	2.4	1.5	2.4	1.4	1.6
22:6	4,7,10,13,16,19	12.7	13.4	8.8	12.5	10.1	14.5	8.7	10.9	13.9	13.7
Total saturated acids		22.3	20.0	23.2	24.9	22.1	25.8	21.8	25.6	20.6	23.0
Total monoenoic acids		46.1	32.3	47.3	33.7	50.1	33.6	45.8	36.6	28.1	24.7
Total polyenoic acids		31.6	47.7	29.5	41.4	27.8	40.6	32.4	37.8	51.3	52.3

[a]The structures of the fatty acids are indicated by the conventional abbreviated nomenclature. The figure preceding the colon gives the number of carbon atoms in the molecule; the figure following the colon specifies the number of double bonds.

[b]Distance of double bonds from carboxyl carbon, which is numbered 1.

For all tissue except skeletal muscle, the content of 16:1 in females was two to four times as high as in males. For the case of ovaries and hepatopancreas this finding might be ascribed to the presence of more depot lipid in the females during oögenesis. Of the five tissues examined, skeletal muscle contained the highest proportions of polyenoic acids. The fatty acid 20:1 was present in notably higher proportions in the hepatopancreas than in other tissues.

Although processing waste from *C. magister* is not available in as great bulk as for king crab and blue crab, some interest exists in its use for the manufacture of by-products. *Cancer magister* offal consists of the carapace, such visceral organs as hepatopancreas and testes, abdomens, and coagulated hemolymph. The present data suggest that the protein content of the offal might be low due to the low protein content of the exoskeleton, and that the amino acid composition might be unbalanced due to the low content of arginine and lysine in the exoskeleton. A study is in progress to assess seasonal fluctuations in the organic and mineral composition of processed *C. magister* offal and meat.

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Monitoring of Fish Tank Oxygen Concentration Using a Digital Recorder

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SCOTT, K. R. 1971. Monitoring of fish tank oxygen concentration using a digital recorder. J. Fish. Res. Bd. Canada 28: 1196–1197.

A method for monitoring fish tank oxygen concentrations at 10-min intervals is described. The millivolt output from a temperature-compensated oxygen meter is connected to a clocked digital datalogger system having a perforated paper tape output. Results from two widely different sets of operating conditions are analysed and automatically plotted using a prewritten computer program.

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OXYGEN concentrations in a fish tank depend on several factors, including water temperature, number of fish, aeration efficiency, and fish activity level. In fisheries laboratories oxygen measurements are usually taken only once a day. Oxygen levels below a certain level are lethal to fish, therefore a system was designed for monitoring oxygen levels at 10-min intervals for a 24-hr period to gain more information about diurnal variations.

Materials and methods — Two separate tests were carried out. Both were conducted in a 1600-liter tank but under widely different conditions. The first test was conducted with a total weight of 70 kg of rainbow trout (*Salmo gairdneri*) having an average weight of 76 g each. The tank temperature was held at 10 C and aeration was accomplished by a full-flow venturi aspirator plus two 0.305-m air stones. The second test was performed with a total weight of 39 kg of rainbow trout having an average weight of 143 g. Tank temperature was 15 C and the venturi aspirator only was used for aeration. In both tests the fish were fed twice, first near the beginning of the test and secondly, just prior to the end. The second feedings were done to collect as much data as possible and to verify any changes in oxygen concentration caused by increased activity or other parameter changes. A Yellow Springs Instrument model 54RC oxygen meter having automatic temperature compensation and an accuracy of 0.1 ppm was used. The probe is a cell having a teflon membrane permeable only to gases and produces electrolytic current proportional to the partial pressure of the oxygen in the water. Cell output is amplified to give a meter reading and a millivolt recorder output. Calibration of the probe in water at 100% air saturation was done in a beaker with an air stone connected to the laboratory air supply.

The probe (Fig. 1A) was mounted on a variable speed laboratory shaker (B) and suspended in the fish tank. The speed of the shaker was adjusted until the meter (C) reached a maximum. The recorder output (D) was then connected to the datalogger input panel (Fig. 2A) and the gain of the preamplifier (B) adjusted until the digital voltmeter (DVM) reading (C) corresponded to the oxygen meter reading. The date of the experiment was entered on the digital thumbwheel switches (D) and the scanner (E) set for a single channel. The pulse interval control (F) was set for 10 min and the system then operated completely automatically with oxygen concentration and time recorded every 10 min. The values were punched on perforated paper tape (G) in binary coded decimal (BCD) code. As a result of continous immersion in the fish tank, there was a drift of approximately 10% in output from the oxygen meter during a 24-hr period caused by the membrane of the probe becoming coated with slime. The meter and datalogger were therefore recalibrated at the end of the experiment and corrected data punched on the tape for subsequent computer analysis. The paper tape data was fed into a remote computer terminal, which transmitted the data via telephone to the IBM 360/65 computer at the University of Manitoba where it was stored on a magnetic disc. The data were then checked for any character or format errors that might be caused by

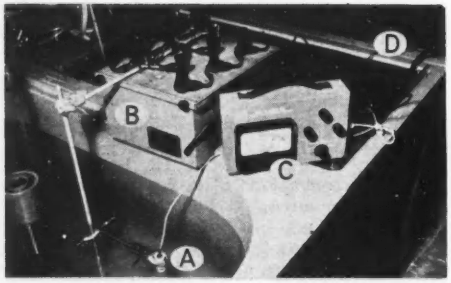

FIG. 1. Oxygen measurement system: A, probe; B, variable speed shaker; C, oxygen meter; D, output to datalogger.

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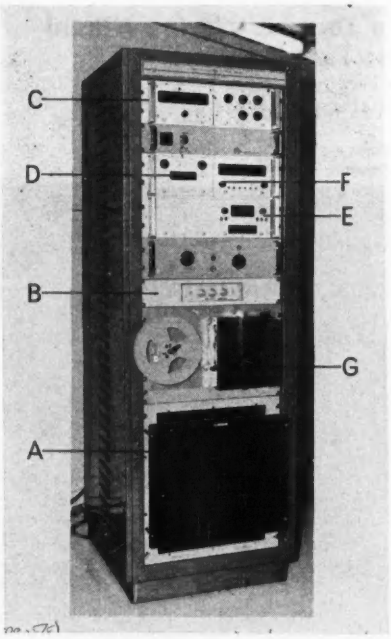

FIG. 2. Digital datalogger: A, input panel; B, preamplifier; C, digital voltmeter; D, thumbwheel switches; E, scanner; F, pulse interval control; G, paper tape punch.

faulty punching or transmission. A Fortran program was written that: (1) adjusted all concentrations to compensate for the calibration drift based on the assumption that the drift was linear with time; (2) converted the time readings to decimal from hours, minutes, second units; and (3) plotted a graph of oxygen concentrations vs. time.

Observations and conclusions — Figure 3 shows the results of two separate experiments. In the first experiment the oxygen concentration decreased in approximately 35 min from 8.4 to 6.8 ppm immediately after feeding of the fish. After this drop, oxygen levels gradually increased to approximately prefeeding levels after about 8 hr. The second feeding period resulted in a similar drop in concentration, although of somewhat larger magnitude, from 9.2 to 6.8 ppm. In the second experiment the oxygen concentration after the initial feeding of the fish dropped from 7.6 to 6.2 ppm in about 45 min. Oxygen levels then gradually

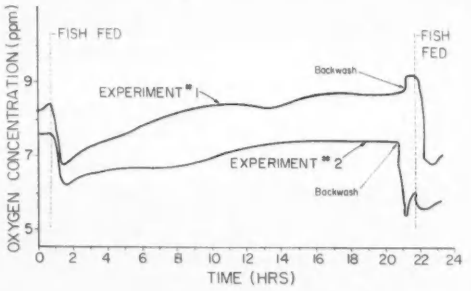

FIG. 3. Variation of fish tank oxygen concentration for two widely different fish populations and operating conditions.

increased somewhat more slowly than in the first experiment, reaching prefeeding levels about 12 hr later. A sudden drop in oxygen level from 7.4 to 5.4 occurred during backwash of two recirculation filters, probably due to malfunction of the aeration devices. After backwash the oxygen level rose to 6.2 ppm, at which time the fish were fed for a second time and the level again dropped to 5.8 ppm in about 15 min. The rapid reduction in oxygen levels after feeding and during other unusual circumstances emphasizes the importance of keeping a margin above the lower lethal level and the advisability of continuous monitoring to ensure that dangerous levels are not approached at any time during the normal maintenance routine. A digital printer with an optional low limit alarm can be attached to the datalogger for quick checking. A group of tanks could be monitored simultaneously for oxygen concentrations by using a number of different meters connected to the datalogger.

Alternatively a system of automatic switching valves could sequentially sample the contents of a group of tanks for pumping to a single oxygen meter. The system combines the accuracy and speed of automatic digital data collection and computation with the convenience of a graphical presentation. The technique can easily be extended to the measurement and analysis of any desired parameters as long as the measuring instrument provides a millivolt output signal that varies with the measured parameter. Corrections or even complete calibration curves can be easily transmitted to the computer centre along with the raw data, and an appropriate computer program will process the data within minutes.

Supernumerary Copulatory Organs in *Dugesia tigrina* (Girard 1850) (Platyhelminthes: Turbellaria)

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BODDINGTON, M. J., AND D. F. METTRICK. Supernumerary copulatory organs in *Dugesia tigrina* (Girard 1850) (Platyhelminthes: Turbellaria). J. Fish. Res. Bd. Canada 28: 1198–1199.

One complete and two incomplete sets of copulatory organs were found in a specimen of *Dugesia tigrina* that had been maintained in the laboratory for 6 months. This is the first record of supernumerary copulatory organs occurring in this species. Their development at a high temperature (23 C) does not support the hypothesis that supernumerary copulatory organs are related to zooid formation.

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IN a stock culture of *Dugesia tigrina* that had been maintained in our laboratory for 6 months, a specimen containing supernumerary copulatory organs was found.

Although such structures have been reported by Kenk (1935) and Okugawa (1955) in other species of *Dugesia*, such abnormalities are rare, and the present report is the first record of supernumerary copulatory organs occurring in *D. tigrina*.

Materials and methods — *Dugesia tigrina* was obtained from Grenadier Pond, High Park, Toronto. The worms were kept in the laboratory at 23 C and fed weekly on beef liver and egg homogenate (equal parts w:w). The tanks were cleaned regularly after feeding.

Worms for histological examination were fixed in Steinmann's fixative, as recommended by Reynoldson (1958), vacuum embedded in 52 C ester wax, sectioned at 8 μ, and stained with Ehrlich's haematoxylin using eosin as a counterstain.

The material has been deposited in the Invertebrate Collection, Royal Ontario Museum, Toronto, Ont.

Observations — A specimen of *D. tigrina*, from five sexually mature animals examined, had one completely developed and two incompletely developed sets of copulatory organs.

The first set of copulatory organs was situated immediately posterior to the pharynx. The second set was situated directly posterior to the first (Fig. 1A), and the third posterior to the second but displaced laterally to the left. The first set was normal in size and the second about three-quarters the size of the first. The third set was less completely developed and smaller than the second (Fig. 1A and B).

The animal had only one pair of mature ovaries, which were no larger than those in other normal specimens. The lateral oviducts led posteriorly and had numerous vitelline glands situated about them. The two oviducts connected in the midline via a short common ovo-vitelline duct, to the bursal canal of the first set of copulatory organs. However, the right oviduct looped upon itself, branched ventrally, and formed a second loop, which connected to the bursal canal of the second set of copulatory organs (Fig. 2).

The bursae of both the first and second sets of copulatory organs were morphologically normal, but differed in size.

Mature testes were present and the vas deferens connected to the bulbar cavities of the penis of the first set of copulatory organs. The second penis was incompletely developed, having only one bulbar cavity, which had no connection with the vas deferens.

The penis of the third set of copulatory organs was small and also contained only one bulbar cavity, which ended blindly at the dorsal extremity of the penis bulb. The male antrum opened into a common antrum and a gonopore was present. The connection of the common and female antra was normal, but the ducts leading from the female antrum were only poorly developed. One duct led posteriorly to a cell mass closely resembling the bursa. This would be abnormal since the bursa is normally anterior to the penis. A second duct led first lateroventrally, then anteriorly, and ended blindly. Although this was obviously an oviduct, it had no connection with the oviducts of the other two sets of copulatory organs.

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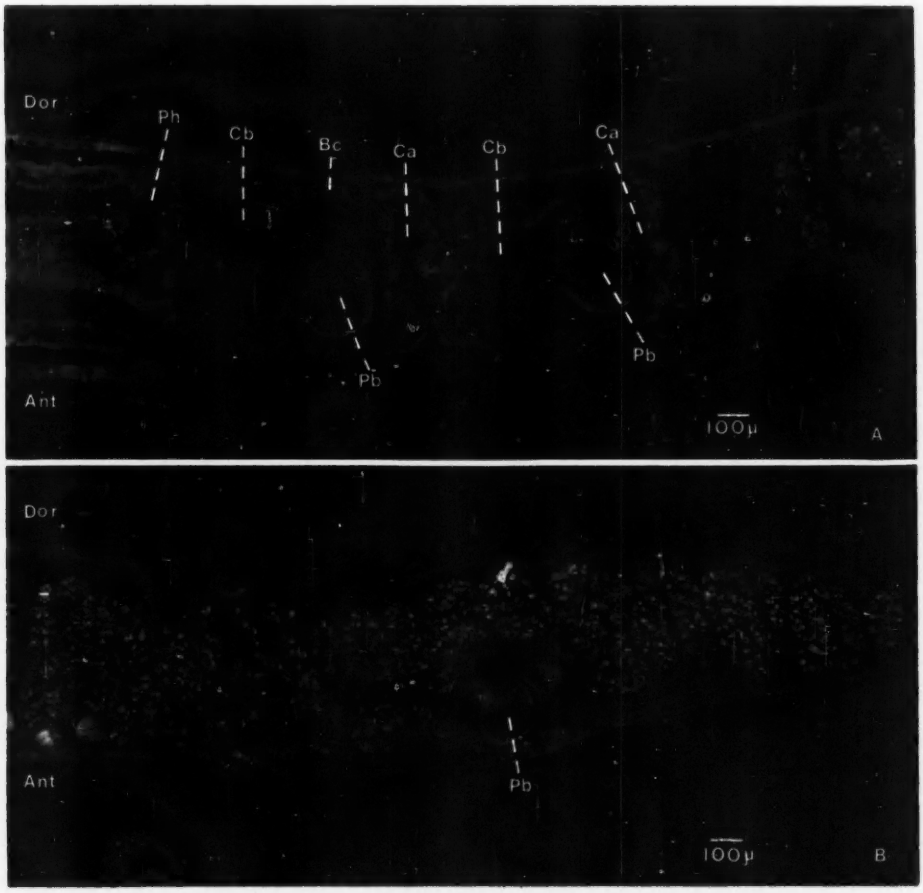

FIG. 1. Parasaggital sections through the copulatory organs in *Dugesia tigrina*. A, through the first and second sets. B, through the third set, situated laterad to the second set. Abbreviations (for Fig. 1 and 2): Ant, anterior; Bc, bursal canal; Ca, common antrum; Cb, copulatory bursa; Dor, dorsal; Fs, false seminal vesicle; Gp, gonopore; Oc, common ovovitelline duct; Ol, left oviduct; Or, right oviduct; Pb, penis; Ph, pharynx; Post, posterior; Vent, ventral; Vd, vas deferens.

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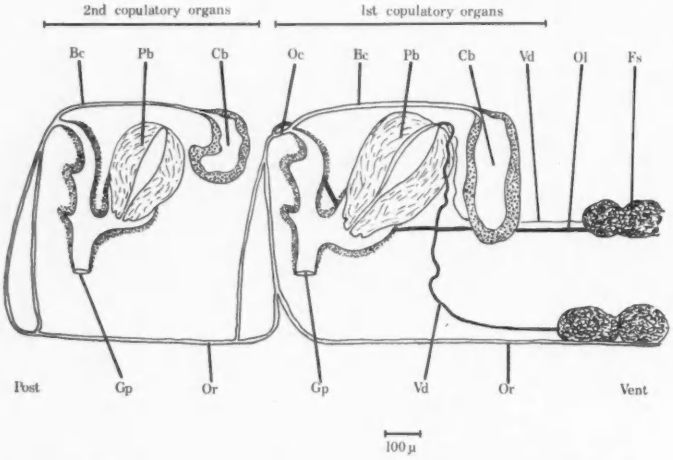

FIG. 2. Schematic representation of the looping of the right oviduct connecting to the first and second sets of copulatory organs in *Dugesia tigrina*. Abbreviations as in Fig. 1.

Discussion — The abnormalities in the copulatory organs of *Dugesia tigrina* have many similarities to those described in *D. dorotocephala* by Kenk (1935) and in *D. japonica* by Okugawa (1955).

Kenk (1935) concluded that in *D. dorotocephala* oviduct formation was directed from the ovary to the bursal canal, but Okugawa (1955) suggested that in *D. japonica* oviduct formation originated from both the bursal canal and the ovary. This latter view is supported by the present observations, as the oviduct from the bursa of the third set of copulatory organs terminated blindly.

Kenk (1935) and Okugawa (1955) considered that the occurrence of supernumerary copulatory organs was due to the formation of a number of zooids at the time of reproductive differentiation. The presence of the zooids was explained by the inhibition of fission at low temperatures (12 C). In the present instance of *D. tigrina* the prevention of fission cannot be attributed to low temperatures, as the worms were maintained at 23 C with varying photoperiod and light intensity.

Under similar conditions, and with the same species, Vowinckel (1970) showed that there was a high rate of fission and advanced gonad development.

Thus, the occurrence of supernumerary copulatory organs in *D. tigrina* cannot be satisfactorily explained by Kenk's (1935) zooid hypothesis.

Acknowledgments — A Student Fellowship from the Fisheries Research Board of Canada to M.J.B. is gratefully acknowledged.

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Paralepis coregonoides borealis (Osteichthyes: Paralepididae) from Davis Strait and Labrador Sea

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Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

LEAR, W. H., AND A. W. MAY. 1971. *Paralepis coregonoides borealis* (Osteichthyes: Paralepididae) from Davis Strait and Labrador Sea. J. Fish. Res. Bd. Canada 28: 1199–1203.

Twenty *Paralepis* specimens were taken from the stomachs of 10 Atlantic salmon (*Salmo salar* L.) caught by drift net at seven fishing stations in the Davis Strait and Labrador Sea. Eleven of the 20 specimens were identified as *Paralepis coregonoides borealis* Reinhardt on the basis of vertebral counts, and the remainder are tentatively assigned to this species. These are the first records of adult specimens from the Davis Strait, though a few have been recorded from the west coast of Greenland.

Received February 3, 1971

EXAMINATION of stomachs of 175 Atlantic salmon (*Salmo salar*), taken by surface drift net of about 3 m depth in the Davis Strait–Labrador Sea area in early autumn of 1970, revealed the presence of 20 *Paralepis* specimens among other food items. The specimens were found in 10 salmon taken in 7 of 17 positions fished in 1970 (Fig. 1), and the number per salmon ranged from one to four. Data on position, date, temperature, and bottom depth where the salmon containing the paralepidids were taken are presented in Table 1.

The fork lengths of the 11 specimens that could be measured ranged from 237 to 285 mm. The head lengths of these 11 ranged from 55 to 62 mm, and the head lengths of eight others ranged from 55 to 66 mm. The remaining specimen, from

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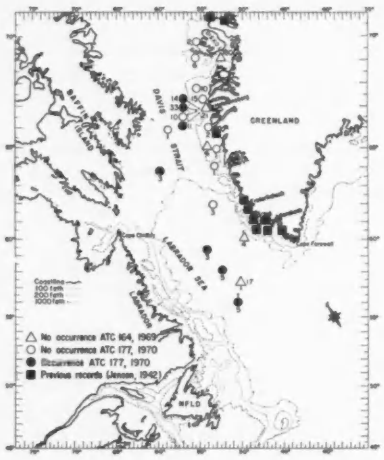

FIG. 1. Area map showing the positions of occurrences and no occurrences of *Paralepis coregonoides borealis* in Atlantic salmon stomachs, numbers of stomachs examined in each locality, and place names mentioned in the text. The positions of previous records (Jensen 1942) are also shown.

which the head was missing, was in the length range 25–28 cm. The fork lengths of the salmon that contained the paralepidids ranged from 63 to 84 cm.

Because of the partly digested state of *Paralepis coregonoides borealis* specimens, it was possible to determine the sex of only 15 of the 20 individuals. These 15 appeared to be females as they contained ovaries with eggs visible under a microscope at 20×. Mead (1960) and Mead et al. (1964) state that various species of the order Iniomi are monoecious and this may be the case for *P. c. borealis*, though there was no evidence that a testis was present. However, the lack of a visible testis may be a result of the partial digestion of each of the specimens.

All but one of the 20 specimens were positioned with head facing anteriorly in the salmon stomachs, thus indicating that the fish had probably been eaten tail first. All the specimens, except those from salmon no. K0648, were the only items contained in the stomachs. In addition to *Paralepis* this salmon contained sand launce (*Ammodytes* sp.), capelin (*Mallotus villosus*), amphipods, and partly digested fish remains. Eleven of the 20 specimens had complete vertebral columns and these were identified as *P. c. borealis* by X-raying the fish and counting the vertebra. The numbers of vertebra (including hypural) are within the range reported by Ege (1930 p. 8) for this species (Table 1). In fact, the vertebral counts overlap with the upper part of the range reported by Rofen (1966) for *Paralepis coregonoides barracudina*, but are within the range for *P. c. borealis*. The average vertebral numbers for *P. c. borealis* reported by Rofen (1966) and in this paper (Fig. 2), 72.611 and 72.636, are not statistically different ($P > 0.90$). Our counts are, however, different from the average of 70.240 (Rofen 1966) for *P. c. barracudina* ($P < 0.01$). Not all the specimens could be identified by this method since some of the fish were partly digested posteriorly. However, since in most instances they occurred in the same stomach with *P. c. borealis* from the same position, and since the heads and anterior parts of the body closely resembled those positively identified as *P. c. borealis*, it was assumed that they were of the same species. Four of the specimens were in good condition and the morphometric proportions, namely predorsal/standard length (60.9–61.6%) head/standard length (22.8–24.7%), and snout/standard length (10.1–11.2%), are all within or very near the range reported by Ege (1930 p. 39) for this species (60.1–61.8, 22.4–24.4, 10.3–11.8 respectively).

Paralepis coregonoides borealis was first recorded in southern Greenland in 1830 at Julianenhaab and in 1831 at Frederikshaab (Reinhardt 1831, 1838). Early records of its distribution are given by Ege (1930) and Jensen (1942), and include a small number of adult records from West Greenland and Iceland, strandings of single fish in southwest England and Norway, and a possible record from the coast of the United States. Ege (1930) also gives the distribution of postlarvae from extensive material collected by the "Dana" expeditions. Apart from a few specimens from off the coast of the United States, the main distribution was between the Bay of Cadiz and southwest Ireland, bounded in the south by the 16-degree isotherm at 150 m.

Paralepis coregonoides borealis is much more common in the northwest Atlantic than the early records indicate, on the basis of reports of its occurrence in the stomachs of various predators. Jensen (1942) referred to its presence in the stomachs of seals off West Greenland. It was reported as plentiful in cod stomachs, in July–August 1960, at about 55–57°N at depths of 225–373 m on the Labrador Shelf (Templeman 1965). *Paralepis coregonoides borealis* was reported from the stomachs of north Atlantic *Alepisaurus* by Haedrich (1964). The species has been recorded from south of Bermuda (Harry 1951). Additional coastal Greenland and northeast Atlantic records, mainly from stomachs

TABLE 1. Catch records of *Paralepis coregonoides borealis* from stomachs of Atlantic salmon taken by surface gillnet in Davis Strait and Labrador Sea, *A T. Cameron*, August 23–October 7, 1970.

Date of capture	Lat	Long	Salmon specimen no.	Bottom depth (*m*)	No. *Paralepis* specimens	Locality	Temp (*C*)			Vertebral no. of complete specimen (including hypural)
							Surface	20 m	50 m	
Sept. 5	63°43′N	59°40′W	K0606	713	3	Davis Strait	4.2	4.1	1.5	72,73,74
5	63 43	59 40	K0608	713	1	Davis Strait	4.2	4.1	1.5	...
16 & 17	66 51	54 59	K0648	73	2	Off Holsteinsborg	3.0	3.0	2.8	74
18	66 47	55 12	K0687	73	4	" "	3.2	3.2	2.5	72,72,72,73
18	66 47	55 12	K0691	73	2	" "	3.2	3.2	2.5	72
22	66 41	54 53	K0727	68	3	" "	2.9	3.0	2.8	...
Oct. 2	59 17	54 10	K0747	2820	1	Labrador Sea	6.0	6.0	6.0	...
3	58 03	52 26	K0761	3330	1	" "	5.6	5.6	4.6	72
4	56 45	50 32	K0765	3500	2	" "	7.0	7.0	4.0	73
4	56 45	50 32	K0774	3500	1	" "	7.0	7.0	4.0	...

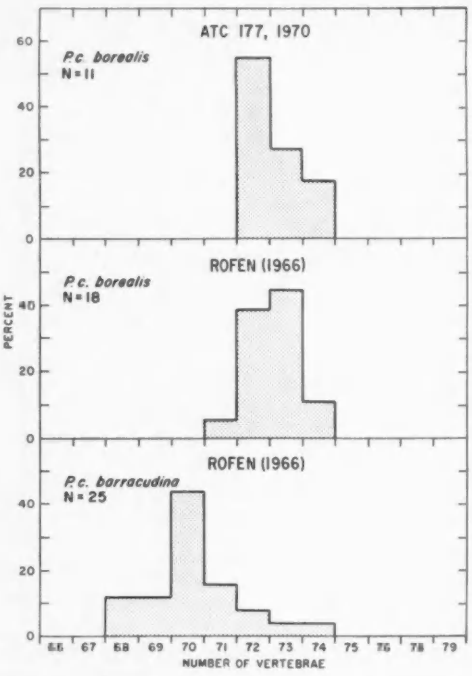

FIG. 2. Vertebral counts of *Paralepis coregonoides borealis* and *P. c. barracudina*, Rofen (1966) and present data.

of predators, are reported in ICES Annales Biologiques from 1947 onwards. Five specimens are reported from east Greenland by Kotthaus and Krefft (1957). Paralepidids, not identified to species, are reported as common in swordfish stomachs off Nova Scotia and New England (Scott and Tibbo 1968). Troyanovski (1966) reports *Paralepis* sp. as the dominant bathypelagic species off central Labrador in June–July.

Templeman (1968) reports 14 specimens of *P. c. borealis* from the stomachs of Atlantic salmon caught in surface drift nets over depths of 2130–3660 m from the southern Labrador Sea, north slope of Flemish Cap, east of the Northeast Newfoundland Shelf, north of the Grand Bank and off the southwest slope of the Grand Bank between March 21 and May 1, 1966. Stomachs of 159 Atlantic salmon tagged as smolts and captured by drift net and set net between Arsuk and Godhavn in West Greenland (Fig. 1) during September–November 1968 and 1969 contained no paralepidids. Also, stomachs of 228 Atlantic salmon caught by drift net in Davis Strait, Disko Bay, and the Labrador Sea during August–September 1969, contained no paralepidids.

The reason for the occurrences of *P. c. borealis* from 1970 stomach samples and lack of them in 1969 samples in relatively the same areas, and also a lack of them in 1968 and 1969 in salmon stomachs from the west coast of Greenland, is a matter for speculation. There was no significant change in water temperatures over the West Greenland fishing banks between October 1968 and 1969 or between March 1969 and 1970 (Meyer and Lenz 1970). The surface temperatures taken during drift-net fishing in the Labrador Sea midway between the Labrador and West Greenland coasts in 1969 and again in 1970 suggest no significant change in surface temperatures for these areas. However, temperatures in the continental slope water off the southern Labrador coast, as revealed by hydrographic sections made by the St. John's Biological Station in August of 1969 and 1970, were higher in 1970 than in 1969. Temperatures in 1969 at depths of 200–1000 m were 3.6–4.0 C; but in 1970 they were 3.7–4.7 C. The species is apparently associated with relatively warm water of 15 C at a depth of 200 m. Ege (1930) gives the southern limit as the 16-degree isotherm at 150 m. Occurrence of the species in the northern Labrador Sea and Davis Strait may be associated with hydrographic warming.

Postlarvae of *P. c. borealis* are known to range vertically from near the surface to about 200 m (Ege 1930). The specimens described in this paper were probably taken near the surface, as salmon are seldom taken on the high seas deeper than the upper 1 or 2 m of drift nets that fish from the surface to 3–5 m below the surface.

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A Simple Field Technique for Obtaining Small Samples of Muscle from Living Fish

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UTHE, J. F. 1971. A simple field technique for obtaining small samples of muscle from living fish. J. Fish. Res. Bd. Canada 28: 1203–1204.

A method for obtaining small samples of muscle from living fish is described. The procedure employs a modified Silverman biopsy needle and samples of approximately 40 mg are obtained. Survival rate in a group of fish held in aquaria and periodically biopsied is excellent. Quantitative mercury analyses were carried out on multiple biopsy samples from a group of fish and the precision of the results is as good as that obtained when analyses were carried out on excised portions of muscle.

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THIS is a description of a rapid technique, readily used in the field, for obtaining small samples of muscle from living fish. It was devised to measure the mercury content of the same fish at various intervals. Though blood samples have been used to monitor various characteristics over a period of time, no feasible method has previously been reported for using other tissues in this way. In a group of fish held in aquaria and periodically biopsied by the new technique, no mortalities have occurred to date.

Technique — A 14-gauge 3½-inch Silverman biopsy needle was modified as illustrated in Fig. 1. The needle was sharpened in three steps, so that when the stylet was in place the unit would move through the skin into the muscle without picking up bits of tissue and foreign matter. Furthermore, the needle itself was sharpened so that it cut into the tissue and tended to force the tissue into the needle. First the needle was flat ground on two opposing sides to give two symmetrically opposed points, which were then bevelled from the outside in,

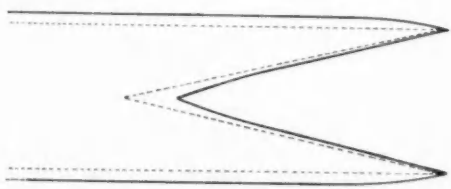

FIG. 1. The modified Silverman needle.

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so that they lay flat on the stylet. Then, with a fine-pointed rotary file, the inner sides of the grooves were sharpened. After this, the sides of the points were sharpened on the inside using the right angle of a fine oil stone. The stylet was cut and bevel sharpened so that when the needle was placed on it, an excessive amount of the stylet did not extend beyond the sharpened end of the needle.

Fish for biopsy were anesthetized with M.S. 222 (ethyl *m*-aminobenzoate methanesulfonic acid salt). A fish for biopsy was then placed in a holding board (Fig. 2), which had been lined with soft foam plastic to limit abrasive damage. The fish was placed along the left side of the unit, giving a curve to the body. The outside portions of the curve above the lateral lines were swabbed with a quaternary ammonium salt as a topical disinfectant. About a half dozen scales were removed and then the sterilized needle with the stylet in place was introduced through the skin into the white muscle parallel to and about 1 inch above the lateral line. The depth was such that when the stylet was removed the end of the needle was in the white muscle. After removal of the stylet, a 30-ml syringe was attached to the needle. The needle and syringe barrel were then driven into the muscle while the syringe plunger was held stationary to supply a slight vacuum. At the end of the plunge, the needle was jerked back slightly to sever the end of the muscle plug. As the needle was withdrawn the vacuum was released. This is similar to the aspiration biopsy technique described by Turner and Sargent (1968) for obtaining samples of human lung tissue. The tissue was then transferred to the bottom of a small polyethylene test tube. The tube was then sealed with heat and the sample frozen and stored over dry ice. The small polyethylene tubes are conveniently made from 4–5-inch sections of polyethylene tubing sealed at the ends. They were cut in half just prior to use. The diameter of the tubing is such to just easily admit the needle. Sealing was accomplished with a portable propane torch and pair of pliers. The needle and stylet were then washed and left in 70% ethanol for a short period. After removal from the ethanol, the needles and stylets were dried and dipped in a tetracyline solution before use. About 400 fish have been processed in 8–10 hr by two workers.

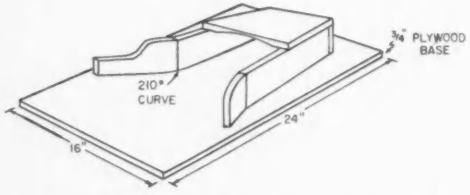

FIG. 2. Board used to hold fish in position for biopsy. The unit is lined with soft foam plastic prior to use.

Comments — Biopsy material obtained in this manner was used to assay the level of mercury present in fish muscle as follows. Samples were weighed by snipping off both ends of the tube, weighing the tube and tissue, transferring the tissue to a digestion flask, and reweighing the tube. The average weight of muscle transferred was slightly less than 40 mg. Three biopsy samples were obtained from each of 12 fish. The amount of mercury present in each sample was determined (Uthe et al. 1970) and a coefficient of variation (100× the standard deviation divided by the mean value) was calculated. From these an average coefficient of variation was calculated and a value of 7.0% was obtained, in comparison with 7.8% for the excised portions of white muscle tissue from the same fish analyzed in the usual manner. Although analysis of portions of white muscle from a single fish showed that the mercury concentration does not vary significantly with the location in the muscle (also see Anon. MS 1968), all biopsy samples were taken from approximately the same location.

As mentioned earlier, fish held in aquaria and biopsied at intervals showed excellent survival rates. After biopsy, these fish were held in clean aquaria, without, feeding for 5 days. No evidence of infection was found in these fish and the wound appeared healed within 2 weeks.

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Two Simple Durable Epifaunal Collectors

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BOURGET, E., AND G. LACROIX. 1971. Two simple durable epifaunal collectors. J. Fish. Res. Bd. Canada 28: 1205–1207.

Vertical and horizontal epifaunal collectors, especially for the infralittoral zone, are described. Special attention is given to stability of the collectors and to easy recovery and replacement of the test panels. The collectors were used under severe environmental conditions and were found very satisfactory. Species collected by the two collectors differed greatly. The vertical collector yielded the greater variety of species, and the horizontal collector the greater number of specimens.

BOURGET, E., AND G. LACROIX. 1971. Two simple durable epifaunal collectors. J. Fish. Res. Bd. Canada 28: 1205–1207.

Les auteurs présentent ici une description d'échantillonneurs épibenthiques de type vertical et horizontal conçus spécialement pour utilisation dans l'étage infralittoral. La stabilité des échantillonneurs, la praticabilité de la récupération et la commodité du remplacement des plaquettes sont les critères qui ont d'abord retenu leur attention. Ces échantillonneurs furent employés avec succès dans des conditions particulièrement rigoureuses. Les espèces épibenthiques récoltées diffèrent beaucoup selon le type d'échantillonneur utilisé: on obtient une plus grande diversité d'espèces au moyen des échantillonneurs verticaux alors que l'on remarque un plus grand nombre d'individus dans le cas des échantillonneurs horizontaux.

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PANEL holders were needed to collect organisms from the infralittoral zone of the St. Lawrence estuary. Settlement panel racks for sessile and sedentary animals were described previously (Coe and Allen 1937; McDougall 1943; Symon-Papyn 1965; Sentz-Braconnot 1966; Cory 1967). These collectors, mostly of the suspended type, were not suitable in high tidal amplitude and strong currents, mainly because of the following objectionable features: (1) depth of collection varies widely according to tidal activity, and (2) heavy algal accumulation about the mooring lines and the collector increases the unit's vulnerability to strong currents and may lead to loss of collectors and submersion of buoys.

The collectors described here are relatively simple. Special attention was given to construction to minimize loss of panels and ensure easy manipulation and recovery.

Description — The vertical collector (Fig. 1) is composed of an iron base (*a*) constructed of four 1 × 5 × 45-cm pieces welded at right angles. The base supports a column (*b*) consisting of a central fixed stem of galvanized iron piping 3 cm in diameter and 53 cm long. A second galvanized pipe 4 cm in diameter and 50 cm high surrounds the stem and supports a 2 × 40 × 80-cm plywood panel holder (*c*) at 15 cm from the bottom by means of two clamps 3 cm wide (*d*).

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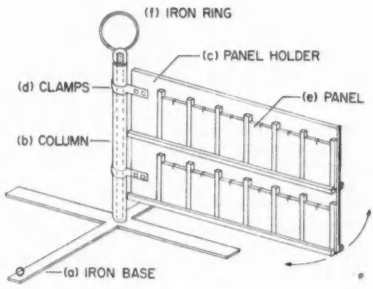

FIG. 1. Diagram of the vertical collector.

The panel holder can rotate so as to offer the least resistance to strong currents. Twenty-four sections 1 × 12 × 18 cm are delimited on it by means of plywood pieces and each section brackets a 10 × 15-cm asbestos panel (*e*). Four wooden cleats with a rabbet 1 cm deep accommodate the bases of the panels, and each panel is fastened to the holder by means of a small brass L-hook. On the free end of the column, a stopper is screwed to the 3-cm threaded upper part, and a 15-cm diam iron ring (*f*) is welded to it. This stopper prevents the movable part of the column from sliding off the stem. The ring is useful for manipulation of the collector and is necessary when lifting the latter out of the water. A small ring is also fitted to the base for tying an anchor to the collector.

The horizontal collector (Fig. 2) is of simpler design. It consists of an iron framework (*a*) supporting a panel holder (*b*). The framework of the 50 × 80-cm base is 1 cm thick and 4 cm wide. This part is surmounted by four rods 35 cm long and 1.5 cm in diameter that support a frame constructed of a 2.5-cm piece of angle iron. The 2 × 50 × 80-cm plywood holder accommodates 16 panels on its underside. Two brass bolts attach it firmly to the framework at one end, while the other end is secured under iron bands 2 cm wide placed diagonally at the corners (*c*). The panels are held in place with brass L-hooks to which plastic identification labels are attached.

Installation — The two types of collectors are anchored (Fig. 3) to prevent displacement. No buoys are attached directly to the collectors, to prevent the accumulation of algae on the mooring line and the high torque that this would cause.

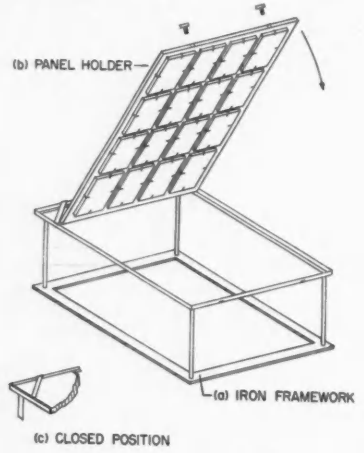

FIG. 2. Diagram of the horizontal collector.

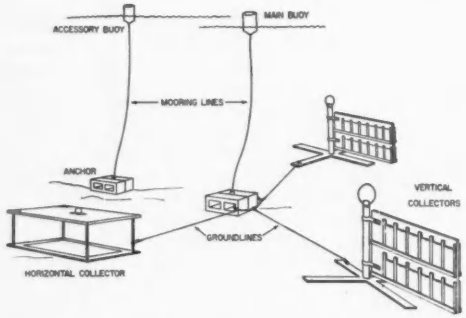

FIG. 3. Diagram of the mooring installation of the collectors.

The anchor, consisting of two concrete building blocks, is fastened to the collectors by means of a 4-m nylon rope with a brass spring clip at each end, providing protection against chafing. A buoy is attached to the anchor and another is placed nearby as a marking point for locating the collectors if the main buoy is lost.

The weight of the collectors (approximately 15 kg) ensures their stability and prevents them from tumbling in currents. Furthermore, the nylon cables are highly reliable, as they are very resistant to biological deterioration; and if the two buoys are lost, and diving is unsuccessful, the groundline offers a greater target when sweeping the bottom. Use of this technique was found highly satisfactory.

Recovery and replacement of panels — The panels are recovered by a scuba diver, who places them in a case with small divisions. When visibility is too poor to work close to the bottom, the diver unfastens the collector, lifts it to the surface with a rope and then recovers and replaces the panels. The inner surfaces and edges of the panels are cleaned, and the panels placed in plastic bags and preserved by immersion in 5% neutral formalin.

Comparative efficiencies and discussion — Vertical collectors were set at four stations (depth ranging from 3 to 5 m at low tide) in the middle and lower St. Lawrence estuary. At one of these stations, horizontal collectors were used simultaneously. Approximately 43 species of invertebrates representing 12 taxonomic groups (Table 1) were collected from the panels on the two types of collectors at this station. Sessile and sedentary groups were the more common on the horizontal collectors, whereas mobile animals were the more abundant on the vertical collectors.

Also, the species collected on the two collectors differed noticeably, since only 14 of the 43 species collected were found on both of them. The greatest difference encountered was among nematodes: only two of the 20 species identified were common to both collector types. Bryozoans were absent from the vertical collectors and gastropods from the horizontal ones. The former group seemed to prefer shaded surfaces, whereas the latter's absence from horizontal panels was probably due to collector position as well as to unfavourable conditions for algal growth.

These data indicate that the vertical collector is the more suitable for study of epibenthic communities as a whole, since it draws a wider range of species. On the other hand, the horizontal collector is preferable if only sessile and sedentary organisms are studied. More sessile and sedentary species gather on the latter and their study is made easier by the absence of algae on these collectors. However,

TABLE 1. Comparative efficiencies of the vertical and horizontal collectors at a station in the St. Lawrence estuary.

Taxa	Vertical collector		Horizontal collector		No. species common to both	Total no. species
	No. species	Maximum no. per dm²	No. species	Maximum no. per dm²		
Foraminifera	Unidentified	4.0(56.0)[b]	–	3.7	–	–
Hydroida	1	+[c]	2	++[d]	1	2
Nematoda	15	260.7	7	8.7	2	20
Bryozoa	0	0	2	2 colonies	0	2
Gastropoda	3(5)[a]	4.7(26.0)	0	0	0	3
Pelecypoda	1	3.7(42.7)	1	2.7	1	1
Polychaeta	2	392.7	4	2661.3	2	4
Ostracoda	3	4.7(11.3)	2	6.7	2	3
Harpacticoida	Unidentified	12343.3	–	2588.7	–	–
Cirripedia	2	4.7(704.0)	2	58.0	2	2
Amphipoda	6	48.7	4	23.3	4	6
Acarina	Unidentified	0(20.0)	–	2.7	–	–
Totals	33		20		14	43

[a]Maximum number of species on vertical panels at other stations.
[b]Maximum density on vertical panels at other stations.
[c]Present.
[d]Abundant.

a complete survey of epibenthic fauna could be conducted only by means of both collectors.

Use of these collectors was very effective in investigations carried out from May to November at the four stations in the St. Lawrence estuary. Over 65 species of invertebrates were taken during the study (Bourget and Lacroix in preparation).

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Killifish "Hemagglutinogens"

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METROKA, C. E., J. V. CHUBA, W. J. KUHNS, R. F. NIGRELLI, AND G. D. RUGGIERI. 1971. Killifish "hemagglutinogens." J. Fish. Res. Bd. Canada 28: 1208–1210.

The "blood-group" activities of mummichog and striped killifish erythrocytes were compared on the basis of monosaccharide agglutination–inhibition tests. Crude wheat germ lectin and some human sera (regardless of A–B–O blood-group status) were shown to contain antikillifish hemagglutinins that were variably inhibitable with N-acetyl-glucosamine and other A–B–O-inactive monosaccharides.

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THIS report deals with the comparative hetero-agglutination and agglutination inhibition of erythrocytes obtained from two species of killifish: the mummichog, *Fundulus heteroclitus* (Linnaeus), and the sympatric striped killifish, *F. majalis* (Walbaum).

Materials and methods — Killifish blood samples were freshly obtained from 10 to 15-g individuals by severing the ventral aorta. Droplets of blood were aspirated into 0.9% saline in the capillary tip of a transfer pipette and diluted immediately with copious quantities of saline. Pooled erythrocyte samples from apparently healthy individuals of the same species were washed three times with saline and made up as 2% test suspensions. Mummichog mucus was obtained from thoroughly saline-rinsed gut mucosa macerated in a small amount of saline and cleared of tissue debris by centrifugation. The supernatant thus obtained was placed in a boiling water bath for 20 min, prior to storage at 4 C.

Boiled primate salivas were provided by the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) of New York University School of Medicine. All of the mammalian inhibitor materials (Table 1) had agglutination-inhibiting potency up to a final dilution of 1:16 or more in reaction mixtures containing an equal volume of 1:8-titer hemagglutinating reagent and homologous indicator erythrocytes.

Reagent sugars for the hemagglutination–inhibition tests were made up as 10% (w/v) stock solutions in phosphate-buffered saline (*p*H 7.3). Crude wheat-germ lectin was prepared from wheat-germ lipase (Nutritional Biochemical Corporation) as described by Liske and Franks (1968). Human group-B serum 30 in Table 1 had an isoagglutination titer of 1:64 with human group A_1 erythrocytes, and human group A serum 222 had an isoagglutination titer of 1:64 with human group B erythrocytes.

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Serological tests were conducted as previously described (Chuba et al. 1968, 1970). Thus hemagglutination–inhibition tests were performed by premixing one drop of potential inhibitor with one drop of hemagglutinating reagent prior to the introduction of one drop of saline-suspended erythrocytes. The tubes were then examined for agglutination following 20–30 min incubation at 21 C, a light spin, and gentle resuspension of the sedimented erythrocytes.

Results — Screening with serum specimens randomly selected from hospital patients and from regular stocks of commercial blood-typing reagents indicated that erythrocytes from both species of killifish were agglutinable with some, but not all, human sera regardless of the A–B–O blood-group status of the serum donor.

As shown in Table 1, for example, human group A serum 222 strongly agglutinated killifish erythrocytes from both species, but human group B serum 30 at a dilution of 1:2 agglutinated only striped killifish erythrocytes. The agglutination-inhibition results in Table 1 further fail to suggest any clearly defined parallelisms between killifish agglutinogens and primate blood-group activities related to the A–B–O system, in which terminal α-N-acetyl-D-galactosaminyl residues are associated with group A specificity and terminal α-D-galactosyl residues are associated with group B specificity (Morgan 1970; Kabat 1970). Moreover (not shown in the table), neither mummichog nor striped killifish erythrocytes were agglutinated by anti-A lectin (lima bean), anti-A_1 lectin (*Dolichos biflorus*), or anti-H lectin (*Ulex europaeus*).

Discussion — The isologous gut-mucus inhibition of the agglutination of mummichog erythrocytes by serum 222 (Table 1) is consistent with the

TABLE 1. Typical hemagglutination and hemagglutination inhibition of erythrocytes of mummichog and striped killifish.

Hemagglutinating reagent	Erythrocytes tested	Reagent titer	Saline (control)	Hemagglutinating reagent mixed with equal volume of: Boiled saliva: Human Group A	Human Group B	Baboon Group A	Baboon Group B	Gastric mucus: Pig Group A	Horse Group B	Mummichog	10% sugar solutions[a]: D-Gal	D-Man	L-Fuc	NAc-Glu	NAc-Gal
Anti-A	Mum'cg	1:1	0[b]	0	0	0	0	0	0	0	0	0	0	0	0
No. 30	Strp. K.	1:4	+++	++	+++	++	+++	+++	+	+++	+++	+++	+++	+++	+++
Anti-B	Mum'cg.	1:4	+++	++	+	++	+++	+	(16)[c]	(4)	++	++	++	(4)	++
No. 222	Strp. K.	1:4	+++	+++	++	++	++	+++	++	++	+++	+++	+++	+++	++
Wheat	Mum'cg.	1:8	+++	-	-	-	-	-	-	-	+++	+++	+++	(16)	+++
germ	Strp. K.	1:8	+++	-	-	-	-	-	-	-	+++	(16)	+++	(8)	+++

[a]D-Gal, D-galactose; D-Man, D-mannose; L-Fuc, L-fucose; NAcGlu, N-acetyl-D-glucosamine; NAcGal, N-acetyl-D-galactosamine.
[b]Macroscopic hemagglutination graded from + to +++; 0 = no detectable agglutination up to 20× magnification.
[c]Numbers enclosed by parentheses = reciprocal of maximum dilution of inhibitor that still completely inhibited macroscopic hemagglutination.

presence of agglutination-inhibiting soluble blood-group substances in other animals (Kabat 1956). Other studies will thus have to consider the possibility that the hemagglutinating activity of killifish erythrocytes may reflect the in vivo acquisition of soluble "killifish-blood-group-substances" by the erythrocyte membranes. In the case of the J blood-group system in cattle, for example, Stone and Irwin (1954) have already shown that animal erythrocytes can acquire blood-group activity as a result of the presence of soluble blood-group substances in various body fluids. There is, in turn, also the possibility that future studies utilizing individual, rather than pooled, samples of killifish blood will unmask significant differences in blood-group activity from one killifish to the next.

The N-acetylglucosamine-inhibitable agglutination of killifish erythrocytes by crude wheat-germ lectin (Table 1) is noteworthy. This lectin has been reported to react preferentially with certain malignant cells and embryonic tissue cells in reactions that are specifically inhibitable with N-acetylglucosamine (Burger and Goldberg 1967; Burger 1968; Moscona 1971). Whether, in turn, the N-acetylglucosamine-inhibitable agglutination of killifish erythrocytes (e.g., the reaction of human serum 222 with mummichog erythrocytes in Table 1) can subsequently be utilized in an indicator system, designed to detect the possible presence of human, antibodies selectively reactive with certain malignant cells, remains to be determined. In the case of the wheat-germ lectin, Liske and Franks (1968) have already reported N-acetylglucosamine-inhibitable cross reactions between wheat-germ lectin and normal mammalian cells. This raises questions regarding the "specificity" of wheat-germ lectin for malignant cells under various testing conditions.

The inhibition of wheat-germ lectin by D-mannose as well as by N-acetylglucosamine in the case of the agglutination of erythrocytes of striped killifish but not mummichog (Table 1) is also noteworthy. This observation suggests that in some cross reactions with certain cell membrane receptors an essential complementary portion of the N-acetylglucosamine-inhibitable combining site can also be blocked by D-mannose molecules. In the case of the agglutination of female chicken erythrocytes positive for the "Hi" factor (by anti-Hi lectin derived from *Pisum arvense* seeds) Scheinberg and Reckel (1965) have similarly reported selective inhibition by D-mannose and other sugars with a D-glucoselike configuration at the 3rd, 4th, and 6th carbons.

The foregoing observation thus (1) further supports the concept that carbohydrate-structured receptors with varying degrees of structural similarity and "blood-group" activity are ubiquitously distributed among the erythrocytes of phylogenetically diverse species of animals (Chuba et al. 1971), and (2) further illustrates the potential usefulness of monosaccharide agglutination–inhibition tests in comparative immunohematology (Chuba et al. 1970; Springer 1970; Wiener 1970).

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Advantages and Limitations of "Simple" Fishery Models in Light of Laboratory Experiments

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"Simple" fishery models as treated herein are those requiring only catch and effort data for fitting. In the models described, the yield-on-biomass curve is considered equivalent to the growth curve of the population: Schaefer, logistic; Fox, Gompertz; Pella and Tomlinson, generalized. The Fox model is more realistic for fish populations than the Schaefer, eliminating the constraint of a symmetrical yield curve. The Pella–Tomlinson model is general, including the others as special instances. Tests against data from laboratory populations of guppies support the validity of the Fox and Pella–Tomlinson models. The Fox model is the simpler to fit of the two (as it has less parameters) and has been shown to be appropriate for a number of commercial fisheries. Advantages of the models include their minimal requirements for basic data and relative ease of fitting. Limitations include lack of provision for reproductive lag and waste of biological information other than catch and effort.

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THE heavy data requirements of many population yield models render the "simple" models (that need only catch and effort) tempting to fishery biologists. Widespread use of such models makes a consideration of their limitations, as well as their advantages, desirable, to the end that proper applications to management can be made. This note will deal with a particular class of "simple" models — those based on relative population growth. My interest in the subject was stimulated by the recent appearance of two contributions to the class: Pella and Tomlinson (1969) and Fox (1970). Data from my fishing experiments on laboratory populations afford an opportunity to compare the models in situations where some of the basic population parameters are better known than in natural populations.

The term "simple" is used here to refer to simplicity in conception of the growth curves underlying the models and to their minimal requirements for basic data. Actually, some of the methods for fitting the models are fairly complex in execution, as a reading of Pella and Tomlinson (1969) will show.

The three models — The models will be discussed in ascending order of generality and complexity, starting with the logistic proposed by Schaefer (1954, 1957). In each model, growth rate is the point of reference. The respective authors equate growth rate (increase in biomass per year) to actual or potential annual equilibrium fishery yield at that size. It is therefore not necessary to discuss yield separately from growth (relative population growth, expressed as $\frac{dP}{dt} \cdot \frac{1}{P}$, where P is biomass and t is age of fish).

The Schaefer model is based on the Verhulst–Pearl logistic curve,

$$\frac{dP}{dt} \cdot \frac{1}{P} = K(P_\infty - P),$$

where K is an empirical constant and P_∞ the upper asymptotic limit of population biomass. The derivative is a parabola, and maximum yield is always at one-half the maximum population size. The Fox model is based on the Gompertz growth curve, which does not require symmetry in the derivative. This constraint of the Schaefer model is thus eliminated, although in its place is the constraint that maximum yield must be proportional to P_∞/e. In simple differential form the Fox model is:

$$\frac{dP}{dt} \cdot \frac{1}{P} = K(\log_e P_\infty - \log_e P).$$

Again K is an empirical constant (equal to K/P_∞ in the Schaefer model), and P_∞ is the upper limit of population biomass. As compared with the Schaefer model, an asymmetrical instead of a symmetrical population growth curve can be fitted. An improved fit (as compared with the

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logistic model) to the Atlantic yellowfin tuna data through 1966 is demonstrated by Fox (1970).

The Pella–Tomlinson model is a generalization, including the other two models as special instances; it can take on an infinite number of forms, including the logistic and Gompertz. In differential form it is expressed:

$$\frac{dP}{dt} \cdot \frac{1}{P} = HP^{M-1}-K.$$

This curve has three constants, H, M, and K, to be calculated in fitting, as compared with two each for the logistic and Gompertz. As pointed out by Pella and Tomlinson, their curve corresponds to the logistic when M = 2, and the limiting form of the derivative as $M \rightarrow 1$ is the Gompertz derivative. Also, since the integral of the basic equation above does not exist when M = 1, Pella and Tomlinson have excluded M = 1 from their calculations, but M may be chosen arbitrarily near one.

Application to experimental data — The experiment of Silliman and Gutsell (1958) provided data on yield from laboratory populations of guppies at four exploitation rates (including zero as one rate). A curve fitted by inspection to the plot of equilibrium yield (equal to growth rate) against biomass (Silliman and Gutsell 1958 fig. 14) shows the relation to be distinctly asymmetrical. The Schaefer model, with its requirement for a symmetrical biomass–yield curve, apparently is not appropriate for these data. Testing against the data, therefore, will be limited to the Fox and Pella–Tomlinson models.

The Fox model was fitted by erecting a regression of the logarithm of catch per unit of effort on fishing effort, the slope and intercept of which provide estimates of the basic parameters (Table 1, line 2). In Fig. 1A the regression is rendered in exponential form for study in more concrete terms. In Fig. 1B the fitted curve to the yield–biomass relation, from the Fox model, is plotted along with the empirical data. A good fit is indicated.

Pella and Tomlinson (1969) also fitted their model (using their "least squares" procedure) to the data from Silliman and Gutsell (1958). They have shown plots comparing calculated and actual data for both population size and catch history. A reasonably good fit was achieved for population size and an excellent fit for catch.

Finally, Fox (personal communication) made additional fits to the guppy results, using data

TABLE 1. Parameters of Fox (1970) and Pella–Tomlinson (1969) models fitted to data from experimental populations of guppies.

	Catchability coefficient	Optimum effort level	Max sustainable yield	Asymptotic population size
Fox notation:	q	X_{opt}	Y_{Emax}	P_{∞}
P & T[a] notation:	q	F_{opt}	C_{max}	P_{max}
Source of estimates		Effective units	g	g
S & G,[b] equilibrium catches, 1951–54 expt, empirical fit, mean population, A & B, fig. 14	.100	3.79	3.65	32.0
Fox model, equilibrium catches, mean population A & B[b]	.100	3.48	3.90	30.4
Fox model, ind. catches, population A,[b] Gulland moving avg[c]	.100	2.80	3.40	32.6
P & T ind. catches:				
Population A,[b] restricted q, min S	.100	2.23	3.49	36.3
Population B,[b] unrestricted q, min S	0.078	2.18	3.36	39.5
Mean	–	2.20	3.42	37.9
Fox model, 1964–65 expt, 1.0 diet, equilibrium catches[d]	.100	3.48	3.55	26.7

[a]Pella and Tomlinson.
[b]Silliman and Gutsell (1958).
[c]Explained in Fox (1970).
[d]Silliman (1968).

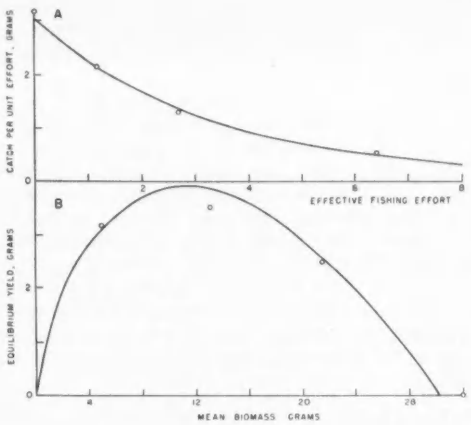

FIG. 1. Curve A: relation between catch per unit of effort and effort, data from mean of two test populations of guppies (Silliman and Gutsell 1958). Curve B: relation of yield to biomass, fitted by model of Fox (1970), with parameters from slope and intercept of curve A in logarithmic form.

from single experiments in the method of Gulland (1968). These data are comparable to those used by Pella and Tomlinson. He also fitted his model to data from a more recent guppy experiment (Silliman 1968).

Parameters from all of the above fits are brought together in Table 1 for comparison. There is generally reasonable agreement among all the estimates of parameters.

Some preliminary evaluations of the models can be made by using the empirical fit of Silliman and Gutsell (top line of Table 1) as a standard. Although such use may be open to question, it at least provides an objective basis of comparison; the Silliman–Gutsell fit was made before either the Fox or Pella–Tomlinson models were available. Of particular interest in the comparisons are optimum effort level and maximum sustainable yield, since these are the data that would be used in administering a real fishery.

For effort level we find that both models are conservative, providing lower estimates than the empirical data in each instance. All three of the estimates for the Fox model are closer to the empirical datum than any of the Pella–Tomlinson estimates. This will be discussed further below.

Estimates from the models bracket the empirical datum for maximum sustainable yield, and all are fairly close to it. There seems to be little choice between the Fox and Pella–Tomlinson models, as far as this parameter is concerned.

Advantages and limitations — The greatest advantage of the "simple" models is, of course, that they require only catch and effort data for fitting. This advantage is not to be taken lightly, for the biological data needed by other models are difficult and sometimes impossible to get. For instance, growth and mortality data must have a time scale, usually determined by assessing the age of individual fish in samples. Aside from the sampling problem, many fish from tropical waters do not lay down the well-marked winter lines in their hard parts (scales, otoliths, etc.) found in temperate zone fish. In contradistinction, catch and effort data are obtainable for almost all commercial fisheries. Most catches are compiled by dealers and by government agencies. Effort data, even though not obtainable from logbook records, can often be estimated with fair accuracy from some product of gear and time (such as boat-months).

Difficulty of the simple models, as fitted by Schaefer and by Pella and Tomlinson, is that they assume instantaneous recruitment. For rapidly reproducing organisms such as bacteria or yeast cells this may be essentially true. Many fish however, first enter the fishery months or years after hatching, and the phenomenon of "reproductive lag" appears. Since simple models do not allow for reproductive lag, they tend to depart from reality in proportion to the age at recruitment of the fish under study. Thus, fish that are recruited at an early age, such as guppies and some tunas, were shown above to be well represented by the simple models. The representation could not be as good, however, for late recruited fish. A further complication (as regards the stock–recruitment relation) is introduced if sexual maturity occurs later than recruitment. Both situations are illustrated by the Pacific halibut, which is recruited at 4 years and matures at 12 (Southward 1968).

For best application, the simple models require a long period of observation. Such long periods introduce the difficulty of getting effort data that are comparable over the entire period, due to the changes in gear type and gear efficiency that frequently occur.

Finally, where biological data of considerable extent have been gathered, the simple models do not make full use of data other than catch and effort. The biologist may ignore such data, but in so doing risks the waste of hard-earned knowledge.

The requirement of the Schaefer model for a symmetrical growth curve has been mentioned above. Although symmetrical growth apparently

is typical of some fishery stocks for some periods, as shown by Schaefer, it demonstrably does not apply to many others. Fortunately, as shown by Fox (1970) and by Pella and Tomlinson (1969), the application of either symmetrical or asymmetrical models to Pacific yellowfin tuna populations produces nearly identical estimates of maximum sustainable yield (although not of other parameters). Thus, if the fishery for this or similar stocks is regulated by a catch quota, the symmetrical model will be practical for estimating the quota. Schaefer (1970) indicated that such a situation also exists for the Peruvian anchovy stocks.

Application of the generalized production model, as fitted by Pella and Tomlinson to data for guppies and yellowfin tuna, led to estimates of catchability coefficients (q) that were considered unreasonable. These results prompted the authors to place constraints on the value of q, restricting it to what they felt to be more realistic values. Imposition of the constraints produced an improved fit to population size history for the guppy stocks, and a question can be legitimately raised as to the validity of the fitting criterion.

Some of the difficulties pointed out in this section were noted by Gulland (1955) and Watt (1956) with respect to the Schaefer model. They did not, of course, have the opportunity of comparison with the Fox and Pella–Tomlinson models or with data from laboratory populations of guppies.

General evaluation — The three models discussed above have made a solid contribution to fishery science. In particular, they represent great insight and ingenuity on the part of the authors in finding models that are applicable when only catch and effort data are available. Some of the models have been used in actual fishery regulation, thus attesting to their practicality. For all the models, it has been shown that they fit data from some real fisheries with considerable fidelity.

Choice among the three models depends both on the nature of population growth in the fishery and on the use to which the parameters from the model are to be put. If the growth (= yield) curve is symmetrical, the Schaefer model will be satisfactory. If results are to be applied only in estimating maximum sustainable yield, the Schaefer model may sometimes be applied even to populations with asymmetrical yield curves. The Fox model comes closer to reality than the Schaefer by eliminating the symmetry requirement, and is simpler to fit than the Pella–Tomlinson. Fox (1970) has shown that his model provides satisfactory fits to data from both Atlantic and Pacific yellowfin tuna. If greater generality is desired, admitting infinitely variable yield curves, the Pella–Tomlinson model is appropriate. This model, as fitted by them, requires a computer searching program that, in some instances, seems to produce unreasonable parameters.

In closing, I would caution that there is no substitute for good biological data. The "simple" models will be valuable in the early stages of an investigation when one desires to make a preliminary appraisal before biological data are available. In other instances they may be put to work if for some reason biological data cannot be obtained. It would be dangerous indeed, however, to ignore all aspects of the population supporting the fishery, except the relation between fishing effort and yield. This is especially true of late recruited and maturing species with a correspondingly large reproductive lag.

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Changes in Blood Lactic Acid Concentrations in Alewives (*Alosa pseudoharengus*) During Passage Through a Pool and Weir Fishway

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Blood lactic acid concentration of anadromous alewives (*Alosa pseudoharengus*) sampled during their upstream migration through a pool and weir fishway was not extremely high (46.7 mg/100 ml) and the degree of exercise exhibited during ascent was judged to be moderate. For fish that were subjected experimentally to severe exercise, the concentration (108.7 mg/100 ml) was more than five times that for rested fish (18.9 mg/100 ml). Of the three groups of fish sampled directly from the fishway pools, only one had an average concentration that differed significantly from that for the rested state.

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THIS study was made to determine the magnitude of changes in the blood lactic acid concentration of the alewife, *Alosa pseudoharengus*, during passage through a pool and weir fishway. The alewife is an abundant anadromous species that crowds into Nova Scotian rivers in May each year to spawn. Fish passage facilities have been provided at many natural and man-made river obstructions for this and other anadromous species.

The effect of fishway ascent on blood lactic acid concentrations has been studied in several species of anadromous salmonids (Collins et al. 1963; and Conner et al. 1964), but similar work on the alewife has not been reported previously (Love 1970).

Materials and methods — The fishway (Fig. 1) is located at the White Rock hydroelectric power development on Gaspereau River, Kings County, Nova Scotia. It contains 65 pools 1.1 m deep, six being 3.0 × 2.4 m, and the remainder 2.4 × 1.8 m. Rest pools, each 6.1 × 3.0 × 1.2 m deep, are located above pools 26 and 51.

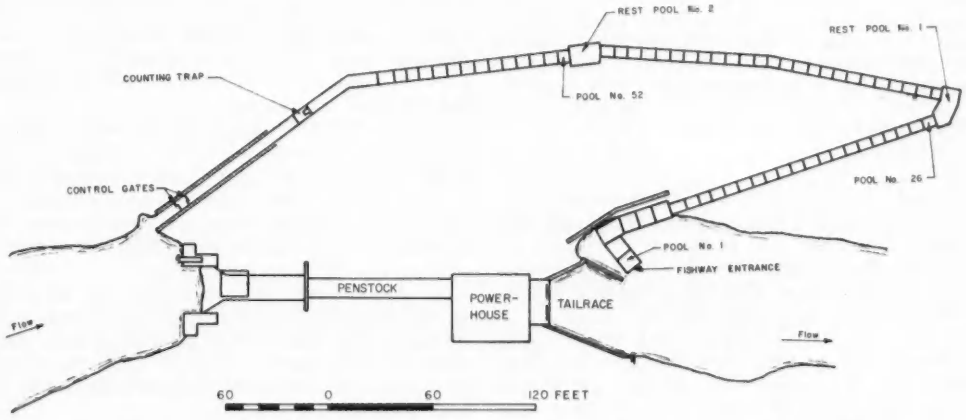

FIG. 1. Plan view of White Rock fishway and hydroelectric power station, Gaspereau River, Kings County, Nova Scotia.

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There is a 0.3-m drop between pools. Each weir has on its downstream end a 0.6-m long chute sloping at 15° to the horizontal. As water passes from one pool to the next, velocity accelerates from 149 cm/sec at the top of each chute to 287 cm/sec at the bottom.

Groups of seven to eight fish were sampled from the tailrace of the power development near the entrance to the fishway and from pools 1 (entrance), 26, and 52 of the fishway. Rested fish were held in quiet water in the trap at the fishway exit for 15 hr prior to sampling. To obtain an indication of the upper limit of blood lactic acid concentration, a group of 20 fish were severely exercised in a confined space (1.8 × 1.2 × 0.2 m deep) by two men who relentlessly chased the fish for 15 min. Several fish died as a result of this treatment and they were excluded from the sample. Fish were sampled on May 25 and 26, 1970. Water temperature ranged from 12 to 14.5 C.

Fish were removed from the water one at a time with a dipnet. Each fish was held ventral side uppermost and within 30–60 sec a blood sample of 2 ml was extracted via cardiac puncture with a heparinized 5-ml syringe lubricated with paraffin oil. The blood sample was transferred into a glass tube and deproteinized in 18 ml of cold 10% trichloracetic acid. After mixing, the filtrate was collected in a 28-cc polyethylene bottle and stored in a frozen state until analyzed for lactate, approximately 3 months later. Blood lactate was determined in the filtrates by the method of Barker and Summerson (1941) and the concentration was expressed as milligrams of lactic acid per 100 ml of whole blood. The total length and sex of each fish was determined. Scale analysis provided an estimate of age.

Standard deviations of the means for total length and blood lactic acid concentrations were calculated for each group of fish. The values obtained for concentrations were transformed to logarithms to reduce the variance. Duncan's 5% multiple range test (Steel and Torrie 1960) was used to compare the logarithmic means. Differences in concentration between males and females under comparable treatment or location were examined using a standard Fisher *t*-test (Simpson et al. 1960) and in all instances found not to be significant at $P = 0.05$. Similarly, lactate concentration and total length were not closely related ($r = -0.25$; $P = 0.10$).

Results — Blood lactic acid concentration in fish taken from pools 1 (entrance) and 52 did not differ significantly from that of rested fish (Table 1), but fish collected from these two pools did possess a significantly ($P < 0.05$) lower lactic acid concentration than the severely exercised group.

Lactic acid concentrations in fish from pool 26 averaged slightly higher than those in fish from the other two pools and differed significantly ($P < 0.05$) from that of the rested group. The concentration in fish from the tailrace of the power development was similar to that of fish from pool 26 and was also significantly different from that of the rested group.

TABLE 1. Mean concentrations of blood lactic acid in alewives, *Alosa pseudoharengus*, according to location in or near the fishway and to treatment. Temperature range 12–14.5 C.

Treatment or location	Age (*years*)	Blood lactic acid (*mg/100 ml*)
Rested after passage[a]	4–6	18.9±20.0[b]
Tailrace	4–5	46.7±32.6
Pool 1	4–5	24.7±15.5
Pool 26	4–6	46.1±23.8
Pool 52	4–5	35.9±28.1
Severely exercised	4–6	108.7±27.3

[a]Seven fish in this test, eight in each of others. Mean lengths from 28.0 ± 1.8 to 29.1 ± 1.2.
[b]One standard deviation.

During passage through the fishway, the concentration of blood lactic acid in alewives increased to approximately 2.4 times that of unexercised fish. In the fish subjected to severe exercise the concentration increased to over five times that of rested fish.

Discussion — The absence of a significant increase in blood lactic acid concentration in fish collected from pools 1 and 52 suggests that passage through the fishway was not a severe exercise for alewives. The significant increase over the rested state in blood lactic acid in fish collected from pool 26 suggests need of a "rest" pool in the first 26 pools of the facility. The value of having two "rest" pools between pools 26 and 52 is indicated by the fact that average lactic acid concentrations decreased from 46.1 mg/100 ml to 35.9 mg/100 ml as the fish ascended the fishway.

Dominy (MS 1971) compared the rate of movement of alewives at pool 1 with that at pool 6 and found that fish moved more rapidly from pool to pool within the fishway rather than at the entrance. This helps to explain the higher concentration of blood lactic acid in alewives from pool 26 compared with that in fish from pool 1.

The observation that the ascent of alewives in this fishway is only a moderate exercise is supported by the work of Collins et al. (1963) who reported simiar results using chinook (*Oncorhynchus tshawytscha*) and sockeye (*O. nerka*) salmon in a pool and weir fishway.

The lactic acid concentration in fish from the tailrace (46.7 mg/100 ml) in comparison with that in fish from pool 1 (24.7 mg/100 ml) suggests that alewives did not enter the fishway directly from

the turbulent water of the tailrace, but rather that they rested in the quiet water on the left bank adjacent to the fishway prior to entering pool 1. Underwater observations at the fishway entrance using scuba reinforced this conclusion in that no fish were seen to enter the fishway directly from the tailrace flow.

The fivefold increase in blood lactic acid from a rested to a severely exercised state is within the customary limits in fish of two- to about 13-fold after exercise as stated by Beamish (1966).

Beamish (1968) reported that the maximum concentration of blood lactic acid in cod occurred 90 min after exercise. The upper limit of lactic acid in the present study was determined using alewives that were sampled within 30 min after exercise. In view of this, the maximum concentration of lactic acid in the blood of alewives may be higher than 108.7 mg/100 ml.

The low level (18.9 mg/100 ml) of blood lactic acid in fish which had ascended the fishway and were subsequently held in quiet water at the fishway exit for 15 hr provided an indication of the degree of recovery from the effects of the exercise. Beamish (1968) reported that for most species lactic acid returned to unexercised levels after 16 hr recovery.

Acknowledgments — Mr G. L. Myatt assisted ably in the field collection and laboratory analysis of the samples. Dr R. H. Cook advised on laboratory procedures. I am grateful to these two individuals as well as to the following who critically reviewed the manuscript: Dr A. Ezzat and Messrs D. B. Lister, C. P. Ruggles, and C. E. Wykes.

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Comment: *Gould's* The case for accurate labeling of superchilled fish

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FREESE, M. 1971. Comment: *Gould's* The case for accurate labeling of superchilled fish. J. Fish. Res. Bd. Canada 28: 1217–1218.

This note comments on a misleading statement in Gould's (J. Fish. Res. Bd. Canada 27: 2101–2103) paper about the cost of instrumentation and the nature of the sample for distinguishing thawed, frozen fish from fresh fish (ultrasonic backscatter method of Freese and Makow (J. Fish. Res. Bd. Canada 25: 605–606)).

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IN a recent *Journal* note, Gould (1970) asserts that the application of the ultrasonic backscatter method (Freese and Makow 1968) to the problem of distinguishing thawed, frozen fish from fresh fish "calls for relatively expensive instrumentation that may not be within the scope of small labora-

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tories. A more important restriction is the requirement for whole tissue, which limits the test to freshly obtained samples." The latter statement is quite misleading. The backscatter method can be used on either whole fish or any small flesh portion provided that it has not suffered excessive mechanical damage. The ultrasonic signature characteristics are not obliterated by further storage subsequent to thawing of the fish.[1] The cost of the equipment is not a significant factor either, since, for example, the sum total of equipment used in our initial experiments can be acquired for about $3500. However, it should be obvious that there are many ways that the ultrasonic backscatter information can be selected (gated, filtered, etc.), analysed, and displayed with the appropriate electronics (unpublished patent application by Freese and Makow in 1968). If the ultrasonic method was to be employed only for determining whether or not a given fish had been frozen, then a variety of relatively simple ultrasonic instruments could be designed for the task.

[1]The changes in the ultrasonic signature are primarily the result of ice crystal cavities that become "fixed" in the tissue to a greatly varying degree, depending on the freezing and storage conditions. The ultrasonic method will thus be ineffective if the degree of fixing is negligible but normally this will not be the case. The fixing phenomenon is a result of the changes in the elastic properties of the tissue components, i.e., changes in the proteins.

FREESE, M., AND D. MAKOW. 1968. Ultrasound backscatter in fresh and thawed animal tissue. J. Fish. Res. Bd. Canada 25: 605–606.

GOULD, E. 1970. The case for accurate labeling of superchilled fish. J. Fish. Res. Bd. Canada 27: 2101–2103.

CORRECTIONS TO RECENT PUBLICATIONS OF THE FISHERIES RESEARCH BOARD OF CANADA

Journal of the Fisheries Research Board of Canada

VOLUME 26, No. 4 — paper by A. O. Dechtiar: *Two new species of monogenetic trematodes (Trematoda: Monogenea) from nasal cavities of catostomid fishes.*

Pages 867 and 868: "500 microns" scales accompanying Fig. 1, 2, and 6, and 7, 8, and 12 should read "50 microns."

Page 869: lines 3 and 4, "0.034" should read "0.069," and "0.027" should read "0.053."

VOLUME 27, No. 7 — paper by A. V. Tyler: *Rates of gastric emptying in young cod.*

Page 1180: Table 1, "A" should read "W_0."

Page 1181: para. 1, line 2, "(Q_{10} = 3.3)" should read "(Q_{10} = 5.5)."

Page 1182: Fig. 3, solid circles represent .13 g, open circles .44 g.

VOLUME 27, No. 8 — paper by J. H. Sparling and C. Nalewajko: *Chemical composition and phytoplankton of lakes in southern Ontario.*

Pages 1412 and 1414: Fig. 5 and 7 are upside-down.

Page 1422: Table 4, footnote, "[a]*Pz* 0.01 significant level = 6.63; ***Pz* 0.05..." should read "[a]***Pz* 0.01 significant level = 6.63; **Pz* 0.05..."

VOLUME 27, No. 10 — paper by S. J. Westrheim: *Survey of rockfishes, especially Pacific ocean perch, in the northeast Pacific Ocean, 1963–66.*

Opposite page 1804: Fig. 11 is upside-down.

VOLUME 27, No. 12 — paper by V. G. Bartnik: *Reproductive isolation between two sympatric dace, Rhinichthys atratulus and R. cataractae, in Manitoba.*

Page 2137: para. 3, line 3, "the male courted the blacknose female" should read "the male courted the longnose female."

VOLUME 28, No. 4 — paper by T. P. T. Evelyn: *First records of vibriosis in Pacific salmon cultured in Canada, and taxonomic status of the responsible bacterium, Vibrio anguillarum.*

Page 522: Table 2, footnote a, line 3, "Data for NCMB from..." should read "Data from NCMB 829 from..."

Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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1549. Bose, R. J., and M. D. Peters. 1971. Occurrence and origin of the tropylium ion in the mass spectra of arylboronic acid esters. Can. J. Chem. 49: 1766–1770. (2)

1550. Sutterlin, A. M., and R. L. Saunders. 1969. Proprioceptors in the gills of teleosts. Can. J. Zool. 47: 1209–1212. (6)

1551. Caddy, J. F. 1969. Development of mantle organs, feeding, and locomotion in postlarval *Macoma balthica* (L.) (Lamellibranchiata). Can. J. Zool. 47: 609–617. (6)

1552. Sprague, J. B., P. F. Elson, and J. R. Duffy. 1971. Decrease in DDT residues in young salmon after forest spraying in New Brunswick. Environ. Pollut. 1: 191–203. (6)

1553. Besch, K. W., and P. Roberts-Pichette. 1970. Effects of mining pollution on vascular plants in the Northwest Miramichi River system. Can. J. Bot. 48: 1647–1656. (6)

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